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Title:Prevention of Ovarian High-Grade Serous Carcinoma by Elucidating Its Early Changes

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14. ABSTRACT: : **Project 1** We will determine the early molecular changes in STIC and their biological significance in developing high-grade serous carcinoma. marker selection and sample preparation will begin in the next coming months. **Project 2** We will evaluate whether the presence of a STIC is associated with different clinical manifestations and/or outcome compare to those patients in whom a STIC was not identified. Molecular profiling will be initiated after quality contorol checking. **Project 3** We will identify the early molecular changes that precede the development of STICs using gene expression analysis of morphologically normal FTE from high-risk women compared to FTE from normal control specimens and use an *in vitro* system and a mouse model to generate a molecularly defined carcinoma resembling HGSC from FTE and OSE using oncogenes expressed in ovarian carcinoma. **Project 4** We plan to if the statin drugs are effective in preventing STIC formation and suppress tumor progression in the OVGP1 mouse model that spontaneously develops STIC and neoplasms.. **Project 5** With the data and cases piling up, we will be able to address the molecular and epidemiologic profile of putative precursor lesions including STIC in the fallopian tubes and ovaries from women at high-risk for ovarian cancer.Also, a pilot study will be performed to determine the most cost-effective way to prepare the tissue sections for studies related to study early tumor development in ovarian cancer. This information will be shared with science community.

15. SUBJECT TERMS
prevention, p53 mutations, high grade serous ovarian cancer and STIC.

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Section I- Purpose and Scope of the Research Effort (all projects)

The purpose of this Ovarian Cancer Consortium is to test the overarching hypothesis that serous tubal intraepithelial carcinoma (STIC) is the precursor and not a metastasis of many, if not most, pelvic high-grade serous carcinomas (HGSCs) but we believe all the other proposed candidates should be investigated in order to determine if STIC is the precursor of all ovarian and pelvic HGSCs or that OSE and CICs harbor precursor lesions as well. Our objective is to then carefully characterize the morphologic, molecular genetic, immunohistochemical (IHC) and epidemiologic features of the precursor lesions(s) (Projects 1-5). If STIC is shown to be the precursor lesion, the data generated by our studies will provide the rationale for our long-term objective, which is the prevention of ovarian HGSC by surgical or medical approaches. Opportunities in the field of cancer prevention have never been greater and therefore our Consortium will undertake innovative studies aimed at providing the scientific underpinning for reducing the burden of ovarian cancer through prevention. Finally, it is important to note that clear cell, endometrioid and mucinous carcinomas are clinically important but they represent only 25 % of all ovarian carcinomas and account for 10% of deaths. In contrast, as noted above, HGSC represents 75% of all ovarian cancers and accounts for 90% of the deaths. Accordingly, we will focus our studies exclusively on the early events associated with HGSC, as it clearly is the most important histologic subtype in terms of frequency and mortality.

The main research efforts in this Consortium are summarized in our five projects.

Project 1: Evaluate whether STICs are precursor lesions and not metastases from a primary ovarian HGSC by analyzing STICs from women with concomitant ovarian HGSCs and determining if the ovarian tumors have acquired additional molecular alterations compared to the STICs which would confirm that STICs are precursor lesions.

Project 2: Evaluate all the proposed site of origin (FTE, OSE, CICs and peritoneum) showing that the morphologic and molecular features of tubal, ovarian and primary peritoneal HGSCs are the same and in conjunction with Project 1 confirming our hypothesis that many, if not most, HGSCs originate in the fimbria and involve the ovary secondarily.

Project 3: Identify the early molecular changes that precede the development of STICs using gene expression analysis of morphologically normal FTE from high-risk women compared to FTE from normal control specimens and use an in vitro system and a mouse model to generate a molecularly defined carcinoma resembling HGSC from FTE and OSE using oncogenes expressed in ovarian carcinoma.

Project 4: Locate and characterize precursor lesions of “ovarian” cancer in a mouse model and explore the role of ovulation and changes in the microenvironment of the ovary and tube in “ovarian” carcinogenesis using human tubal xenografts in nude mice.

Project 5: Determine the molecular and epidemiologic profile of putative precursor lesions in the fallopian tubes and ovaries from women at high-risk for ovarian cancer. In addition, Project 5 will determine if these biomarkers and associated precursor lesions are modifiable by oral contraceptives (OCPs) or anti-inflammatory agents as OCPs in particular are known to prevent ovarian cancer and impact survival.

Section II, III and IV (5 projects are reported here individually)

Before the recent progress is summarized, we would like to update the list of publications related to this consortium.

Publications supported by DoD Ovarian Cancer Consortium (OCPR: W81XWH-11-2-0230)

Title: Prevention of Ovarian High-Grade Serous Carcinoma by Elucidating Its Early Change

2011-Current

1. George SH, Greenaway J, Milea A, Clary V, Shaw S, Sharma M, Virtanen C, Shaw PA: Identification of abrogated pathways in fallopian tube epithelium from BRCA1 mutation carriers. J Pathol 2011, 225:106-17 PMID: 21744340.

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3. Kurman RJ, Vang R, Junge J, Hannibal CG, Kjaer SK, Shih Ie M: Papillary tubal hyperplasia: the putative precursor of ovarian atypical proliferative (borderline) serous tumors, noninvasive implants, and endosalpingiosis. Am J Surg Pathol 2011, 35:1605-14 PMCID: PMC3193599

4. Visvanathan K, Vang R, Shaw P, Gross A, Soslow R, Parkash V, Shih Ie M, Kurman RJ: Diagnosis of serous tubal intraepithelial carcinoma based on morphologic and immunohistochemical features: a reproducibility study. Am J Surg Pathol 2011, 35:1766-75. PMCID: PMC4612640

5. Kuhn E, Kurman RJ, Sehdev AS, Shih IM: Ki-67 labeling index as an adjunct in the diagnosis of serous tubal intraepithelial carcinoma. Int J Gyn Pathol 2012, 31:416-22. PMCID: PMC3715095

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10. Tone AA, Virtanen C, Shaw P, Brown TJ: Prolonged postovulatory proinflammatory signaling in the fallopian tube epithelium may be mediated through a BRCA1/DAB2 axis. Clin Cancer Res 2012, 18:4334-44. PMID: 22753593

11. George SH, Milea A, Shaw P: Proliferation in the normal FTE is a hallmark of the follicular phase not BRCA mutation status. Clin Cancer Res 2012, 18:6199-207. PMID: 22967960

12. Tian Y, Chen L, Zhang B, Zhang Z, Yu G, Clarke R, Xuan J, Shih IM, Wang Y: Genomic and network analysis to study the origin of ovarian cancer. Systems biomedicine 2013, 1:55-64.

13. Kuhn E, Ayhan A, Shih Ie M, Seidman JD, Kurman RJ: Ovarian Brenner tumour: a morphologic and immunohistochemical analysis suggesting an origin from fallopian tube epithelium. Eur J Cancer 2013, 49:3839-49. PMID: 24012099

14. Vang R, Shih Ie M, Kurman RJ: Fallopian tube precursors of ovarian low- and high-grade serous neoplasms. Histopathology 2013, 62:44-58. PMID: 23240669

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16. Ardighieri L, Lonardi S, Moratto D, Facchetti F, Shih IM, Vermi W, Kurman RJ: Characterization of the immune cell repertoire in the normal fallopian tube. Int J Gyn Pathol 2014, 33:581-91. PMCID: PMC4617751.

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Intraepithelial Carcinoma and Ovarian Carcinoma in Mouse Models. Clin Cancer Res 2015, 21:4625-62. PMCID: PMC4609247.

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30. Jung JG, Shih IM, Park JT, Gerry E, Kim TH, Ayhan A, Handshuh K, Davidson B, Fader AN, Selleri L, Wang TL. The expression of PBX1, a stem cell reprogramming factor, in ovarian cancer chemoresistance. Cancer Res, in press. PMID: 27590741

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Project 1: Evaluate whether STICs are precursor lesions and not metastases from a primary ovarian HGSC by analyzing STICs from women with concomitant ovarian HGSCs and determining if the ovarian tumors have acquired additional molecular alterations compared to the STICs which would confirm that STICs are precursor lesions.
Research site: Johns Hopkins University
Project Leader: Ie-Ming Shih
Co-investigators: Doug Levine (NYU), Robert J. Kurman (JHU)

Section II. Progress to Date

Task 1. Determine the clonal relationship and tumor progression pathway from STIC to invasive high-grade serous carcinoma (HGSC).

Task 1a. Case selection and sample preparation including LCM, DNA extraction (1-20 months)
Progress: We have collected a sufficient number of cases for this task.

Task 1b. TP53 mutational analysis of the potential precursor lesions of HGSC (8-24 months).
Progress: The task has been completed and the data have been published PMID:21990067. So we will not reiterate the progress here. Of note, this paper has been highly cited (more than 140 times according to Google Scholar) since publication.

Task 1c. Allelic imbalance assay by digital SNP analysis and data analysis (24-36 months)
Progress: This task has been modified and the results have been published (see below).

Kuhn E, Wang TL, Doberstein K, Bahadirli-Talbott A, Ayhan A, Sehdev S, Drapkin R, Kurman RJ, Shih IM. CCNE1 amplification and centrosome number abnormality in serous tubal intraepithelial carcinoma- further evidence supporting its role as a precursor of ovarian high-grade serous carcinoma. Mod Pathol, 29:1254-1261, 2016.

Task 2. Determine the early molecular changes associated with serous tubal intraepithelial lesions.

Task 2a. Immunohistochemistry study on ovarian cancer-associated markers on STICs and other putative precursor lesions (18-40 months)
Progress: This task has been completed. The progress is that we have identified at least 6 highly specific ovarian cancer-associated markers on STICs including LAMC1, CCNE1, topoisomerase II, RSF-1, TET1, and loss of ALDH1A1.

Task 2b. In situ hybridization and/or mRNA expression analysis on those markers that the antibodies are not available (30-40 months)
Progress: As discussed in the last report, we have been fortunate to identify good antibodies (laminin C1, ALDH1A1, p53, topoisomerase II, cyclin E1, etc.) for immunostaining purposes and there is no need for us to consider *in situ* hybridization at this moment.

Task 2c. Verification of new markers from Project 3 in precursor lesions (24-56 months)
Progress: This task has been completed and the results have been published in the following journals including J Pathol (PMID: 23378270), Am J Surg Pathol (PMID: 22892598) and Mod Pathology (PMID: 25216223).

Task 2d. Telomere FISH on STICs and other precursor lesions (36-48 months)
Progress: This part of study has been completed as previously discussed.

Task 2e. Data analysis and preparation for publications (40-60 months)
Progress: We will perform data analysis and prepare the results for publication for the new sequencing study involving STIC and its related tubal lesions.

Section III. Problem Areas for Project 1

There are no potential areas needed to be discussed at this moment as we continue making progress as expected.

Section IV. Future Works in Project 1

As discussed in the last progress report, we have finished the main tasks except the Task 1 (To determine the clonal relationship and tumor progression pathway from STIC to invasive high-grade serous carcinoma) because of the technical issues challenging the analysis of extremely minute tissues (and scant genomic DNA) from STIC, the precursor lesion of high-grade ovarian serous carcinoma. These technical issues were discussed in previous progress reports. Nowadays, we are very excited to know about the newly developed technique that can potentially overcome those issues thanks to the recent advances in DNA purification and library preparation that are just becoming available very recently. This new method, although expensive, would allow us to stay with the original plan to micro-dissect a variety of tubal precursors and perform next-generation sequencing on STIC, STIL and p53 signatures. In some cases, we will further compare the genomic landscape between tubal precursors and adjacent carcinoma as proposed in the Task 1. The results from this study performed during the no cost extension period will give us, for the first time, cogent evidence to support the central hypothesis of this OCRP consortium project that most of ovarian high-grade serous carcinomas are derived from fallopian tube lesions. The expected result will significantly impact on the ovarian cancer research field and have profound translational implications (early detection and prevention) for patients in years to come.

Project 2: The relationship between serous tubal intraepithelial carcinoma and invasive pelvic serous carcinoma

1. INTRODUCTION:

Ovarian cancer has traditionally been thought to develop from the OSE or cortical inclusion cysts, but recent data suggest that a majority of advanced HGSC may originate from the fallopian tube epithelium. Although highly provocative, this hypothesis requires further validation and therefore we propose to analyze a large group of pelvic, which includes ovarian, tubal and primary peritoneal, HGSCs diagnosed using traditional criteria with and without STICs in women whose fallopian tubes have been processed using the SEE-FIM technique, currently the most comprehensive method of evaluating fallopian tube epithelium. Our primary objective is to determine whether there are subsets of HGSC, which have different molecular profiles and different clinical behavior based on their presumed site of origin or whether there are no differences and that they are essentially the same irrespective of their site of origin. We will also compare the molecular profiles of normal tissues to HGSCs as a whole and HGSCs with and without STICs placing specific emphasis on the ovarian surface and distal fallopian tube epithelium.

2. KEYWORDS: Serous tubal intra-epithelial carcinoma, ovarain cancer, ovarian carcinoma, high-grade serous carcinoma, serous, STIC, in-situ cancer, SEE-FIM, ovarian surface epithelium, fallopian tube, genomic profiling, molecular, genomics.

3. ACCOMPLISHMENTS:
MAJOR GOALS:
Task 1: Determine the frequency of STICs in patients with advanced pelvic HGSC.

Task 2: Evaluate whether the presence of a STIC is associated with different clinical manifestations and /or outcome compare to those patients in whom a STIC was not identified.

Task 3: Compare the molecular features of advanced pelvic HGSCs with and without associated STIC to various normal pelvic tissues.

ACCOMPLISHEMENTS UNDER THESE GOALS (FOR THIS REPORTING PERIOD):
Task 1: Complete – nothing to report for this period.

Task 2: Complete – nothing to report for this period.

Task 3: Compare the molecular features of advanced pelvic HGSCs with and without associated STIC to various normal pelvic tissues.

Task 3a. Collect 100 HGSC specimens that had SEE-FIM processing from Consortium sites; 10-20 specimens per site with the balance contributed from MSKCC (Completed previously)

Task 3b. Collect 10 normal tissues from each of 5 anatomic sites; 50 total tissues. Tissues to be collected across Consortium sites, with each site contributing at least 2 of each normal tissue type with the balance contributed from MSKCC (Completed previously)

Task 3c. Process all specimens on various genomic platforms

All tumor specimens had been processed during previous reporting periods. We had also been able to finalize quality control (QC) on our normal tissues, which had been challenging due to small cellular quantities obtained from anatomical brushings. We developed a pooling strategy such that at least 3 pools (of 3-5 patient specimens per pool) from each normal anatomic site generated sufficient material to perform RNA sequencing. The RNA sequencing on these normal samples pools have now been completed.

Task 3d. Analyze data on each platform according to proposal.

1. Major activity – basic analysis of tumor samples is complete for each platform.
2. Specific objectives: To better understand the molecular etiology of HGSCs, we report a multi-center integrated genomic analysis of advanced stage tumors with and without STIC lesions.
3. Significant results: (Results presented in prior reporting period) Unsupervised cluster analysis of miRNA data failed to identify a separation between cases with and without STIC lesions. The most significant focal DNA somatic copy number alterations were shared between cases with and without STIC lesions. Class comparison of the RNA sequencing data using the 9,236 most variable genes identified 69 differentially expressed genes at $P < 0.01$, none of which passed multiple comparison correction. We were able to reproduce the established gene expression clusters for high-grade serous ovarian carcinoma as previously published and demonstrated [PMID: 21720365, 18698038, 23257362]. Among these cases we did not see a difference in progression-free survival. We also identified multiple regions of focal amplification and deletion that appear similar between tumors with and without STIC lesions.

Conclusions: The data suggest that the molecular features of HGSCs with and without associated STIC lesions are mostly shared, indicating a common biologic origin among all cases. We propose that HGSC originates in the distal fallopian tube and a STIC lesion is only identifiable in ~50% of cases prior to intraperitoneal shedding of malignant cells.

(New results for this reporting period)

Since the last reporting period, we have been working on performing the barcode analyses between the normal sample pools and the tumor specimens. This has been a heavily computational process requiring frequent interactions between the NYU site and the biostatistical core at JHU. Figure 4 shows that the fallopian tube sample pools cluster separately, and that the ovarian surface and peritoneum cluster together as would be expected for similar-type mesothelial tissues. Figure 5 shows that the tumor samples cluster most closely with the fallopian tube normal pools. Figure 6 shows the specific correlations on individual samples basis indicating that most cluster closest to the fallopian tube normal pools.

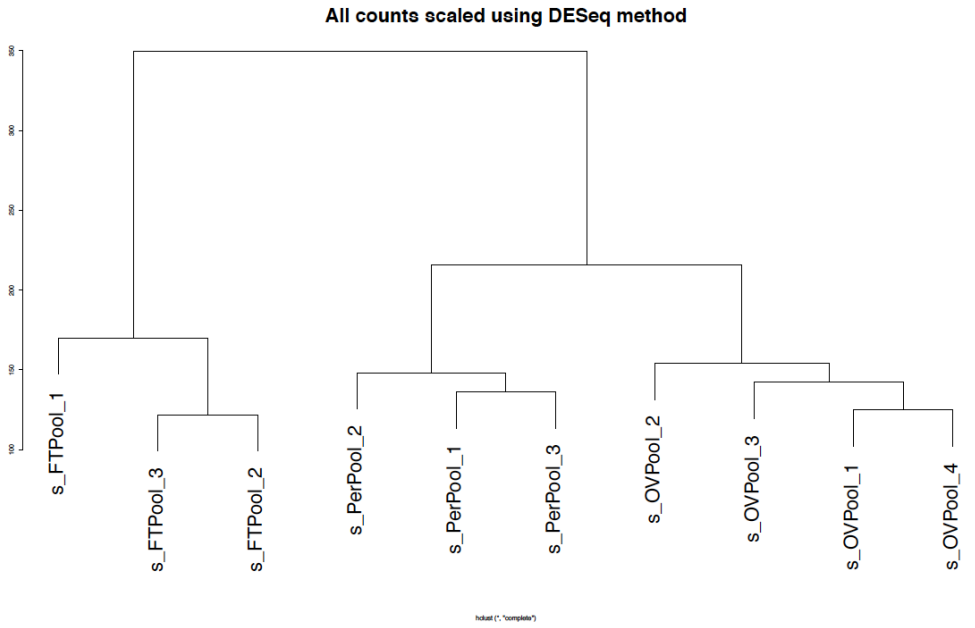


Figure 4. The mRNA expression profile for each normal sample was clustered using unsupervised hierarchical clustering of the most differentially expressed genes.

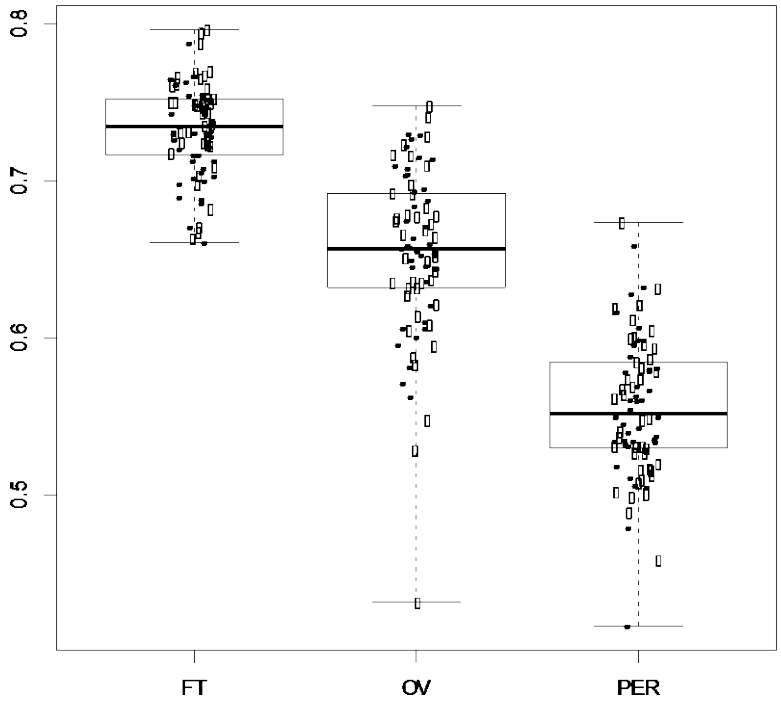


Figure 5. The mRNA expression profile for each tumor sample was correlated to prototype profiles derived from normal fallopian tube, ovarian and peritoneum tissue, respectively, using genes most differentially expressed across normal tissue types. The presence of STIC lesions is indicated with solid circles.

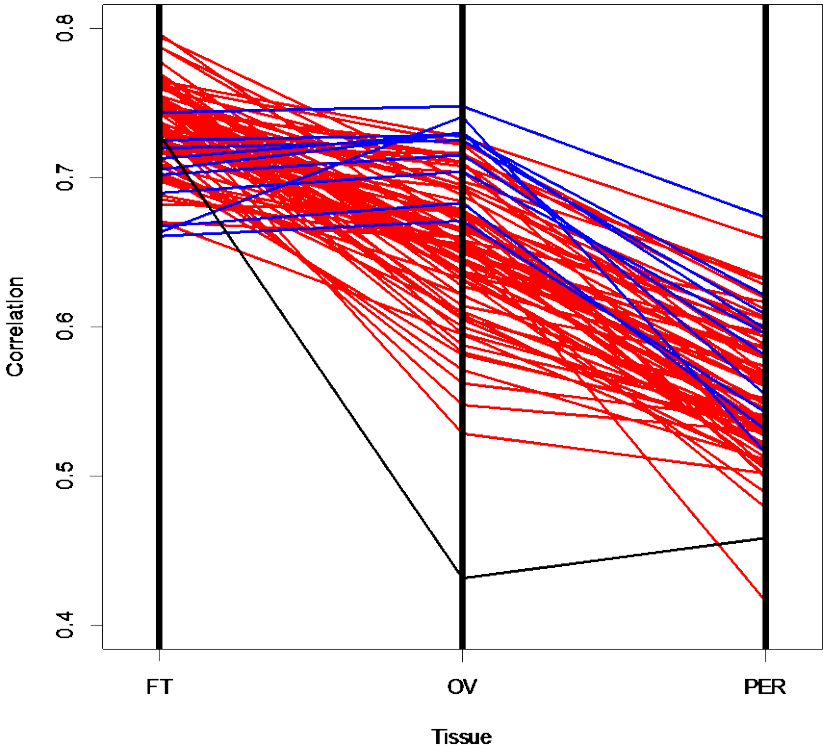


Figure 6. Each line corresponds to a tumor samples. Seventy-five of eighty-five tumor samples (88%) were most closely correlated with the normal fallopian tube phenotype (red and black lines). The remaining 10 (blue lines) more closely resembled the normal ovarian tissue. Normal peritoneum tissue had the lowest correlation with all but one sample (black line).

Other achievements: None to report

TRAINING AND PROFESSIONAL DEVELOPMENT: Nothing to Report.
DISSEMINATION TO COMMUNITIES OF INTEREST: A manuscript draft has been circulated to co-authors.
PLANS DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH GOALS: We will have submitted a manuscript by the next reporting period, as one has already been circulated to the co-authors.
We have also identified several microRNAs that appear to be differentially expressed between tumors with and without STIC lesions. There was no proposed work to validate these findings. In the no cost extension period, we propose to validate these findings with independent samples that are archival (FFPE) and not frozen, as was the case with the original set of specimens. We will use the nanostring platform to determine the gene expression of these select microRNAs in a cohort of validation specimens. In this manner, we can determine if these differentially expressed microRNAs may be associated with ovarian cancers that do or do not have identifiable STIC lesions.

4. IMPACT:

IMPACT ON THE DEVELOPMENT OF THE PRINCIPAL DISCIPLINE: These results are expected to make an impact on the understanding of ovarian cancer tumorigenesis. The outstanding question in our principal discipline is whether or not all high-grade serous carcinomas develop from the distal fallopian tube through serous tubal intra-epithelial carcinomas. These data and results will support the notion that nearly all high-grade serous carcinomas do develop from the distal fallopian tube through serous tubal intra-epithelial carcinomas since little genomic variation has been found between tumors with and without serous tubal intra-epithelial carcinoma. These data will ultimately help to shape prevention and early detection approached for ovarian cancer.

IMPACT ON OTHER DISCIPLINES: Nothing to report.

IMPACT ON TECHNOLOGY TRANSFER: The data will afffect the development of technology used for early detection of ovarian cancer considering that devices will need to be developed for interrogation of the distal fallopian tube.

IMPACT ON SOCIETY BEYOND SCIENCE AND TECHNOLOGY: The findings will help to educate the society about the origins of ovarian cancer and encourage women throughout the world to request bilateral salpingectomy (removal of both fallopian tubes) instead of bilateral tubal ligation as a measure to prevent unwanted pregnancy. The data is also likely to result in a greater role for bilateral salpingectomy at the time of hysterectomy with ovarian preservation.

5. CHANGES/PROBLEMS:

CHANGES IN APPROACH: None noted.

ACTUAL OR ANTICIPATED PROBLEMS OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM: We had some delays in collecting and processing the normal tissue samples due to the limited cellular contents obtianed from fresh intra-operative brushings. We were able to overcome

this problem through a pooling approach that is statistically sound and eliminates batch effects among the normal samples.

CHANGES THAT HAD A SIGNIFICANT IMPACT ON EXPENDITURES: There was an accounting error at MSKCC that resulted in approximately $130,000 being charged to this account during the wrong budgeting period. This was a result from a failure to properly encumber anticipated and approved charges. During this reporting period we have had to review all expenses from prior years and cost transfer money from other funds to cover this shortfall.

OTHER CHANGES: There were no other changes to any human subjects, vertebrate animals or biohazard concerns.

6. PRODUCTS:

PUBLICATIONS, CONFERENCE PAPERS, AND PRESENTATIONS: Abstract presentation at the 2015 Annual Meeting of the Society of Gynecologic Oncology.

OTHER PRODUCTS: Genomic data will be publicly deposited during the next reporting period.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

INDIVIDUALS WHO HAVE WORKED ON THE PROJECT: Douglas A. Levine, Maria Bisogna, Narciso Olvera, Fanny Dao – No change.

CHANGE IN THE ACTIVE OTHER SUPPORT OF THE PD/PI(S) OR SENIOR/KEY PERSONNEL: Douglas A. Levine received a leadership award from the DOD to serve as Assistant Dean of the Ovarian Cancer Academy.

W81XWH-15-1-0428 (OC140582) (PI: Levine)
Congressionally Directed Medical Research Programs 9/30/2015 - 9/29/2020
The Ovarian Cancer Academy: A Team-based Science Approach
This project will help to plan the scientific and leadership aspects of the Ovarian Cancer Academy. It will also assist all Academy members with project design, grant review and interpretation of results.

During the past year of the award, Douglas Levine, moved jobs from Memorial Sloan-Kettering Cancer Center where he was an Attending Surgeon and Head of the Gynecology Research Laboratory to New York University Perlmutter Cancer Center where he is Director of the Division of Gynecologic Oncology, Head of the Gynecology Research Laboratory, and Professor of Obstetrics and Gynecology. This move occurred on May 13, 2016 and for this reason, his report spans the time frame from September 30, 2015 to May 13, 2016. The move did not affect any of the objectives or scope as they relate to the statement of work or the objectives of the award.

OTHER ORGANIZATIONS INVOLVED AS PARTNERS: Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS: Nothing to report.

9. APPENDICES: None.

PROJECT 3. Identification of molecular changes preceding STICs in FTE from high-risk women using in vitro and in vivo models

Investigators: PI - Shaw, Shih

Research site: University of Toronto

1. **INTRODUCTION:**

In this project we proposed to determine the expression profiles of anatomically high risk FTE (fimbrial) from women at high genetic risk (BRCA1 mutation carriers) compared to the FTE profiles from women at low risk of High Grade Serous Carcinoma (HGSC), and we propose that these changes may play key roles in the earliest events of serous carcinogenesis. To this end, we will use a molecularly defined system to sequentially express ovarian cancer-associated genes including those identified in this project into ovarian surface epithelium (OSE) as well as fimbrial FTE to determine a) if FTE is more prone to neoplastic transformation and b) if the FTE-derived tumors more closely simulate HGSC than OSE-derived tumors.

Recently described precursors of HGSC, the p53 signature, a latent precursor, and Serous Tubal Intraepithelial Carcinoma, a pre-malignant precursor, occur most frequently at the distal and fimbriated end of the fallopian tube (FTE). We recently demonstrated that the FTE of BRCA1 mutation carriers, at genetic risk of HGSC, have altered signaling pathways compared to controls. A key question is whether the gene expression differences identified at the ampulla between BRCA1 and non- mutation carriers is similar to differences at the fimbria. This study determines the transcriptome profiles of normal fimbrial FTE and normal ampulla FTE which may lead to insight of why the distal end of the fallopian tube is preferentially predisposed to malignant transformation

Specific Aim 1. *Detect and select genes differentially expressed in morphologically normal fimbrial FTE from women at high genetic risk of HGSC.*

Specific Aim 2. *Model alterations associated with normal FTE from high-risk women and STIC in vitro and in vivo.*

2. **KEYWORDS:** *STIC, FTE, HGSC (High Grade Serous Carcinoma), BRCA, GSTA2, CEBPD, xenograft, modelling*

3. **ACCOMPLISHMENTS:**

The purpose of this Project is to identify the early molecular changes that precede the development of STICs using gene expression analysis of morphologically normal FTE from high-risk women compared to FTE from normal control specimens and use an *in vitro* system and a mouse model to generate a molecularly defined carcinoma resembling HGSC from FTE and OSE using oncogenes expressed in ovarian carcinoma. Project 3 consists of several tasks listed below:

Task 1. Establish expression profiles of fallopian tube epithelium from *BRCA1* mutation carriers and controls, and of serous cancers in mutation carriers.

Progress: To date, we have collected and processed over 200 formalin-fixed and/or cryopreserved cases of fallopian tube and fimbriae specimens. These cases include samples from BRCA1 and BRCA2 mutation carriers undergoing prophylactic surgery, patients undergoing debulking surgery for High Grade Serous Carcinoma, and patients undergoing salpingo-oophorectomy for non-malignant

reasons. An integral aspect of Project 3 *Specific Aim 1* is to determine the relationship between hormonal response and BRCA mutation status in the normal fallopian tube epithelium. As a result, a significant effort has been placed on determining the menstrual status of samples collected – this included reviewing the endometrium of corresponding samples when available. We completed histological reviews of fallopian tubes from BRCA1 and BRCA2 mutation carriers, along with matching controls and cancers and identified 84 cases that were eligible for gene expression profiling. One of the key questions within this aim is whether the gene expression differences identified at the ampulla between BRCA1 and non-BRCA1 mutation carriers is similar to differences at the distal end of the fallopian tube – the fimbria.

To best answer the ampulla versus fimbria conundrum, and perform a technically robust experiment, we used cryopreserved ampulla and fimbria from non-BRCA mutation carriers with known ovulation cycle status.

A). Micro-dissection of selected cryopreserved tissue samples

Completed June 2016, manuscript preparation

Background*:* Recently described precursors of high-grade serous carcinoma (HGSC), the p53 signature, a latent precursor, and Serous Tubal Intraepithelial Carcinoma (STIC), a pre-malignant precursor, occur most frequently at the distal and fimbriated end of the fallopian tube (FTE). In 3 previous reports, we have demonstrated that the FTE of BRCA1 mutation carriers, at genetic risk of HGSC, have altered signaling pathways compared to controls. Ovarian production of ROS is released after the LH surge to induce ovulation. Reactive oxygen species (ROS) have been implicated in serous carcinogenesis. The objective of this study is to compare the transcriptome profiles of normal fimbria (high-risk epithelia prone to transformation) FTE and normal ampulla (low-risk epithelia) FTE which may lead to understanding the distal end of the fallopian tube as the preferential anatomic location of the fallopian to tube for cellular transformation.

Methods*:* Snap-frozen matched fimbria and ampulla tissues were controlled for age and ovarian cycle status. Cases included 12 luteal phase and 12 follicular phase women at no known risk for ovarian cancer. Laser capture microscopy was used to micro-dissect FTE cells, using 7-10 sections per case. Total RNA was isolated, RNA extracted and cDNA amplified. The expression profiles were generated using Affymetrix Human Genome HTA-2.0 Array. 5um sections of the FFPE specimen of the profiled cases were stained for Ki67, p53, CK7 and GSTA2

Results/Developments*:* Using gene level differential expression analysis with the Affymetrix Expression Console software, we performed unsupervised hierarchical clustering analysis with all 24 samples. We used a fold change of < -2 or > 2 and ANOVA p-value < 0.05 as a cut-off criteria for selecting genes. The cases clustered predominantly by ovarian cycle status rather than by their differences in anatomical origin or their matched pair. There were 427 genes differentially expressed amongst the 4 groups – Fim-Luteal, Fim-Follicular, Amp-Luteal and Amp-Follicular. Independent of ovarian cycle status, very few differences (35 genes – SALL1, SERPINA3, ANXA13, PDK4, ME1, GSTA1, GSTA2 – genes involved in metabolic pathways) were observed between the ampulla and fimbria FTE. The epithelia of the anatomically high-risk fallopian tube – the fimbria, show few differences in gene expression profiles compared to the lower risk portion – the ampulla. Expression differences predominantly are in response to the hormonal milieu, i.e. the secretory and proliferative phases of the ovarian cycle. The increased anatomic risk of the fimbria is likely due to effects of the microenvironment, such as repeated exposure to follicular fluid at ovulation, rather than intrinsic differences of the FTE in the two sites. We have validated the expression of glutathione S-transferase A2 (GSTA2) in both fimbria and ampulla using IHC. The expression array data had shown a

difference of 2.1 fold increase in the fimbria. This is recapitulated in fimbria and ampulla (of the same case) with IHC (Figure 1A-C).

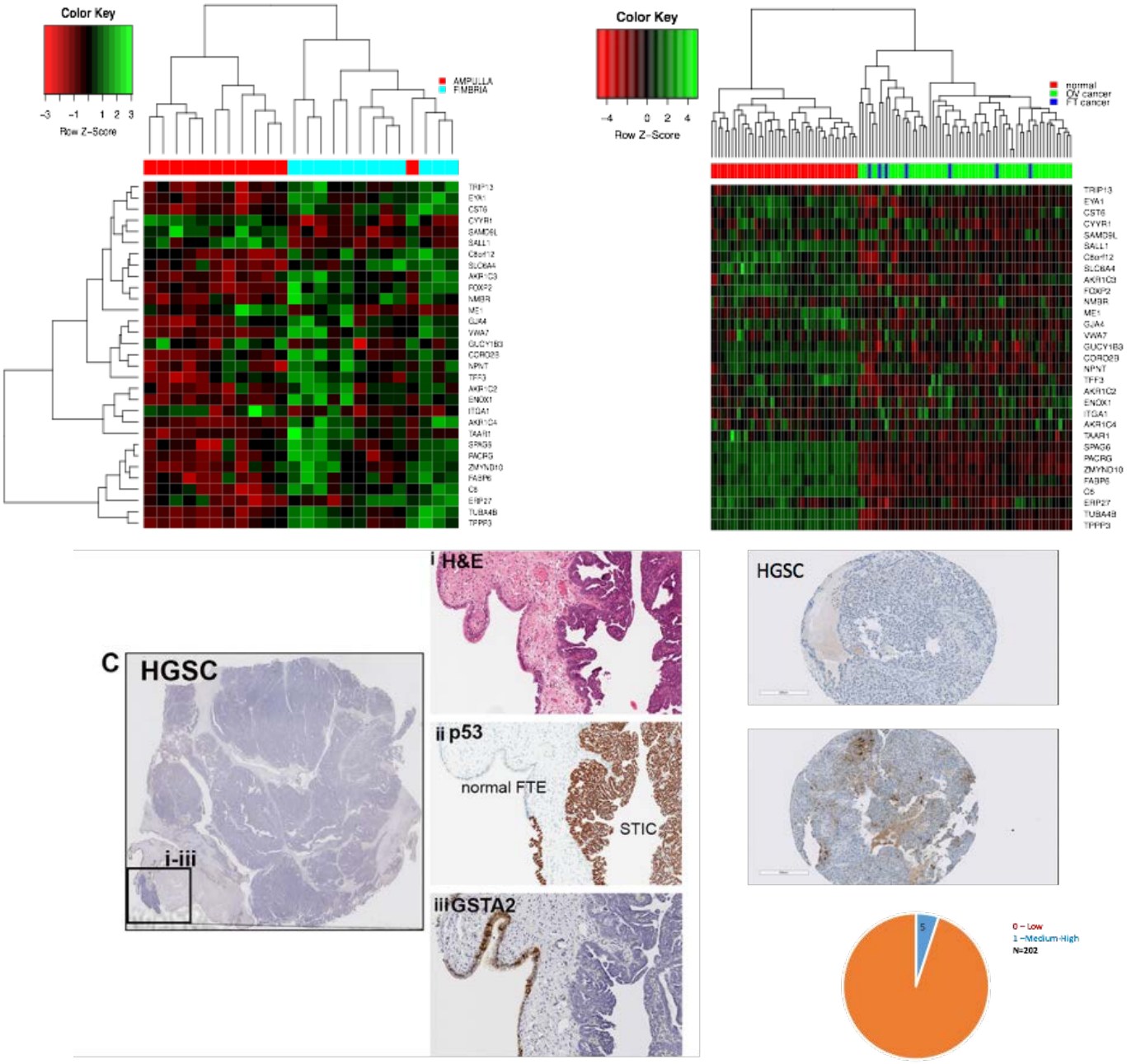


Figure 1: A-C. **A.** Differential expression of genes between the high-risk fimbria and ampulla. **B.** This gene signature can also segregate normal FTE from fallopian tube cancer and HGSC. **C.** There are more GSTA2 positive cells in the fimbria compared to the ampulla. Decrease in expression of GSTA2 in a fallopian tube epithelial cells which over-express p53. Expression of GSTA2 in high-grade serous cases is markedly reduced. 95% of HGSC cases in TMA show a decrease in this antioxidant enzyme.

B). Microdissection of selected paraffin tissue samples

LCM of samples completed, awaiting bioinformatic analyses (70%)

Summary/Developments: We have completed laser capture micro-dissection (LCM) on fimbriae from formalin-fixed paraffin embedded (FFPE, processed by SEE-FIM protocol) out of the proposed 68 cases; we have performed LCM on 68 cases which includes 9 HGSC (germline BRCA1 mutation) cases. We have RNA-sequenced 68/68 cases which are waiting for subsequent bioinformatics analysis. Below is a summary of FFPE cases that have had LCM performed and sequence completed. Refer to Appendix 1 for a complete list of case.

Methods: FFPE tissue samples were sectioned at 10um and placed onto Pen-membrane slides (Leica). Sections were then cut using a Laser Capture Microdissection microscope (Leica). Approximately 6-12 sections were cut per case depending on surface area of epithelium available. Sections were stored in lysis buffer, snap-frozen and stored at –80 degrees until RNA extraction. RNA was extracted using the Roche High Pure FFPE Micro Kit (Roche). Samples were stored at –80 and submitted to the sequencing facility for RNA quantification and RNA sequencing. These samples are prepared by using Illumina Tru-Seq Stranded Total RNA kit with Ribo Goldready for the Illumina Hi-seq 2000 V3.

We are in the process of analyzing RNA sequencing data and will validate genes when results are available

Task 2*.* Validate mRNA and protein expression of selected classifier genes

Progress: In preparation for targets from the genomic profiling of fimbrial gene expression arrays, we are in the process of creating one additional cancer TMA containing a set of 300 HGSC samples with known family history of breast/ovarian cancer and patient clinical history including debulking status, treatment, recurrence and overall survival. This TMA will be useful in assessing the alteration of chosen targets within a larger set of cancers with different family history of cancer, as well as assessing the impact of such targets on clinical outcome. We have begun accruing cases to build the TMA. Pathological examination of each HGSC case is also being performed prior to building the TMA. As the microarray on the FFPE samples have not yet begun, the validations of targets in both normal FTE and STIC are delayed. We submitted all the micro-dissected FFPE RNA for analysis.

We are awaiting the results from the FFPE project. The TMA is in the process of being built

We used genes that were differentially expressed between normal FTE and high-grade serous cancer (LKB1/STK11- published Oncogene 2015), ampulla derived differences between BRCA1 mutation carriers and non-carriers (Rb, p16, CD3, CD8, CD68 and CEBPD) and ampulla versus fimbria derived gene expression differences (GSTA2 and other antioxidant enzymes) to study and validate classifier genes. In addition, we studied the hormonal induced gene signatures between and amongst these groups of experiments. We have subsequently validated and characterized the role of these genes in normal fallopian tube tissue and STIC cases. Our results suggest novel roles of these genes in the context of the development of ovarian cancer.

To study the expression profile of these different genes, we constructed 2 fallopian tube tissue microarrays and 3 high grade serous TMAs (PT2, HGSC2011, HGSC4). In addition, we are creating an additional high grade serous TMA – 90% completed (all cases have been selected and organized and we are awaiting the building of TMA blocks).

Task 3. Model alterations associated with high-risk tubal epithelium and tubal intraepithelial carcinomas, in vitro.

We have established and propagated FTE cells and characterized it using western blots analysis, growth curves and immunocytochemistry staining. Transformed FTE and OSE cell lines were injected into mice in 2015/2016. Over the past year we have generated multiple tumors and are in the process of characterizing each to determine if candidate alterations modulate in-vivo malignant phenotypes and if there are differences between malignant phenotypes of FTE and OSE, and FTE-BRCA1/2 and OSE-BRCA1/2 origins. Copy number alterations and gene expression arrays will be performed on these tumors. Histological staining will require that we provide antibodies and utilize core pathological services to carry out stains.

Summary - Immortalized and transformed cell lines from both FTE and OSE, cells derived from both BRCA1 mutation carriers and non-carriers. In particular, 16 OSE cell lines have been created –3 from BRCA1 carriers – iOSE390F, iOSE267F, iOSE592F (F=familial ovarian cancer) and non-carriers – iOSE120 and iOSE523. Cell lines were transfected with hTERT (ht) and SV40, and either vectors over-expressing cMYC, hRASV12 or PIK3CA-H1047R.

In vitro assays were performed to compare proliferation, anchorage-independent growth and invasion between 2 BRCA1 (iOSE 267F and iOSE 592F) and 2 non-BRCA cell line over expressing hTERT and SV40 and either cMYC or hRASV12. All 12 lines showed proliferation and anchorage-independent growth. 8 of these lines with cMYC or hRASV12 were then injected in NSG mice (NOD.Cg-Prkdcscid). All injections with these cell lines were done in the mammary fat pad of 6 week old female mice. We are currently in the process of monitoring the mice and performing regular measurements of tumors that arise.

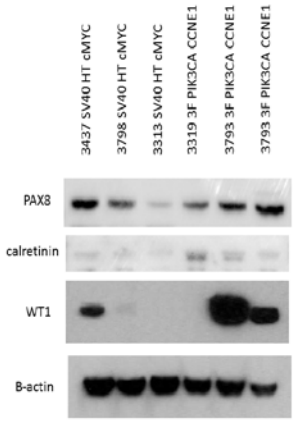
Similarly, 16 FTE lines were generated from BRCA1 carriers - FTE3793 and FTE3798, BRCA2 carrier - FTE3313 and non-BRCA carriers – FTE3437, FTE3619 and FTE63857 over-expressing hTERT and SV40 and either cMYC or hRASV12. As with iOSE lines, 8 of the FTE lines have been injected in the mammary fat pad of NSG mice and we are currently monitoring the mice for tumour development. In addition to FTE cells with SV40 hTERT, FTE cell lines – FTE3793, FTE3313, FTE3319 and FTE63857 were generated with 3F (TP53-R175H, E7, and hTERT).

The parental immortal lines of 4 cases were karyotyped: FTE-3437 and FTE-3619 (controls); FTE-3313 (BRCA2 mutant) and FTE-3798 (BRCA1 mutant). The FTE BRCA mutant cell lines demonstrated varying types of tetraploidy whilst the control (BRCA wild type) demonstrated had fewer tetraploid chromosomes. It is hypothesized that SV40 alone is sufficient to stimulate genomic instability in the FTE cells.

These lines were subsequently transfected with either PIK3CA-H1047R, HRASV12, cMYC and/or CCNE1 and injected into NSG mice at the mammary fat pad.

Methods – Cell lines were generated and grown in tissue culture. At approximately 80% confluency, cells were trypsinized, counted and suspended in matrigel. Cells were injected within the mammary fat pad and the intraperitoneal region of female 6 week old NSG mice. Mice are monitored twice a week for signs of tumor development and are sacked when tumor has reached 1.5 cm. Mice injected intraperitoneally are monitored twice a week along with a weight check performed at the end of each week. Mice are sacked if the abdomen is distended or mice exhibit signs of lethargy and malaise (indicated by hunched posture and raised fur). Mouse dissection involves removing the tumor, liver, spleen, lungs and intestines which are then embedded in paraffin for histological analysis. Tumor sections are also snap-frozen for subsequent genomic analysis.

Results/Developments - To date, tumors have developed from mice injected with ovarian epithelial surface cells and fallopian tube epithelium cells at the mammary fat pad and the intraperitoneal cavity. FTE cells injected intraperitoneally have produced ascites in conjunction with tumors that line the abdominal cavity and major organs. Multiple tumor nodules have been found around the uterine



horn and within the mesocolon.

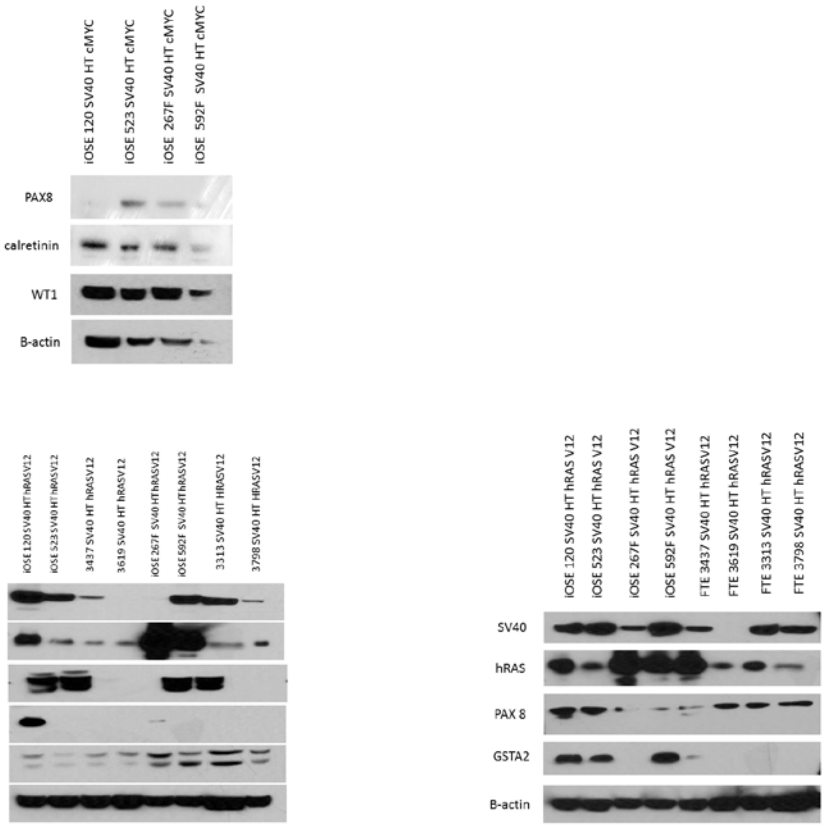


Figure 2. A. immortalized OSE and FTE cells were transformed with a combination CCNE1, hRASv12, cMYC and PIK3CA H1047R. These blots represent characterization of protein expression of transformed the cell lines.

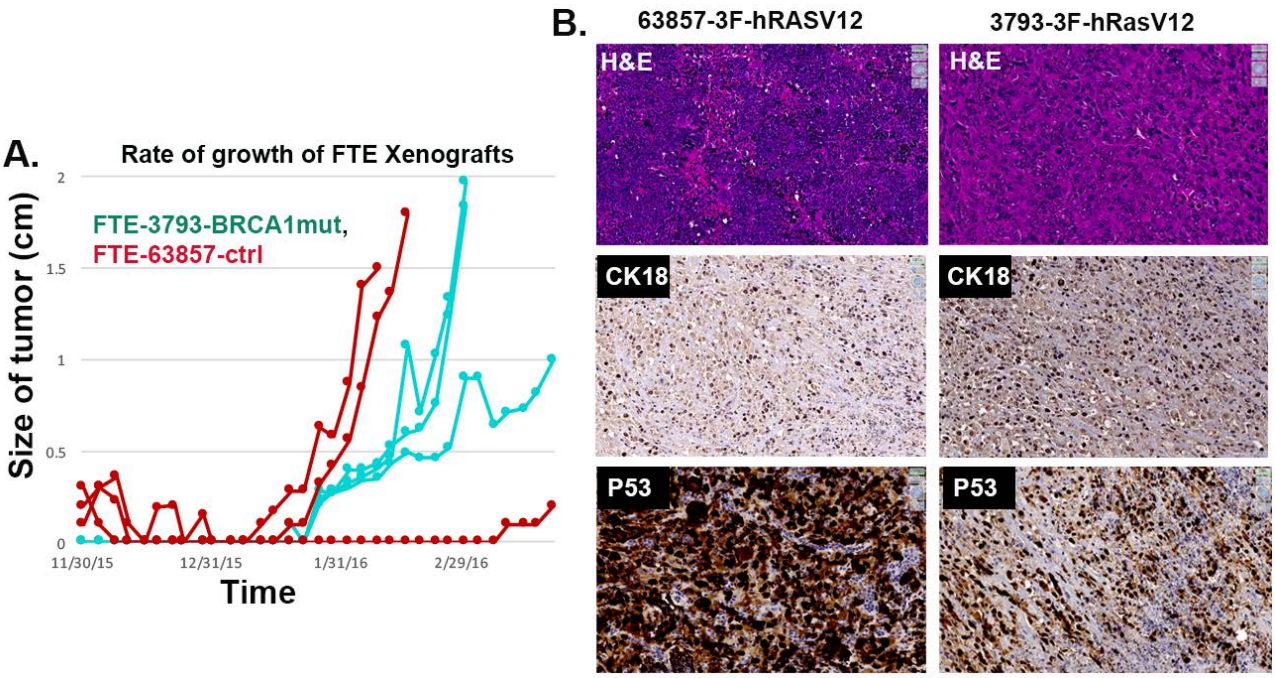
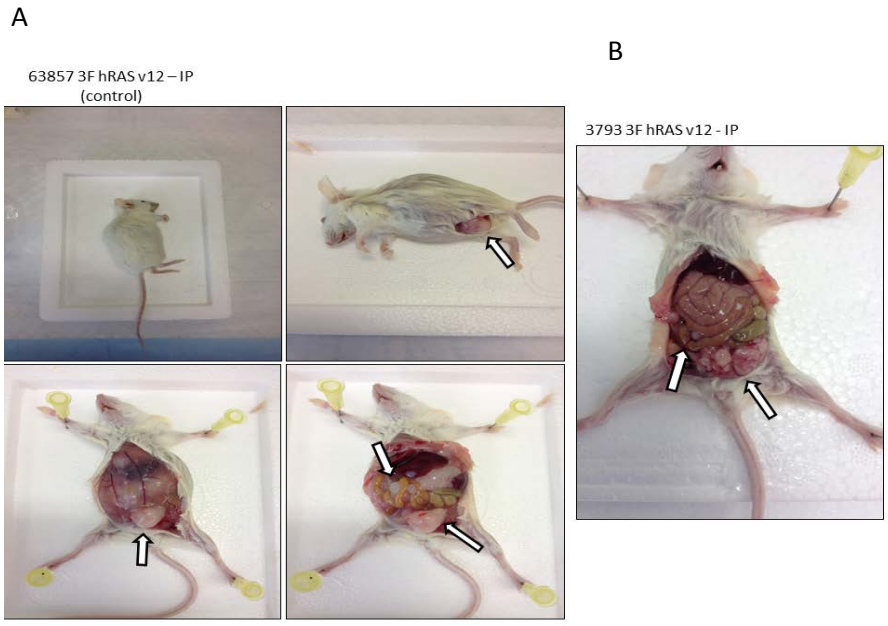


Figure 3. A. Growth curve of FTE derived xenografts. FTE cells were transformed with mutant p53-R175H, E7, hTERT and hRASV12. FTE-3793 BRCA1(4239delAG), FTE-63857 wild type BRCA1. **B.** Xenografts of both transformed FTE express CK18 and p53.



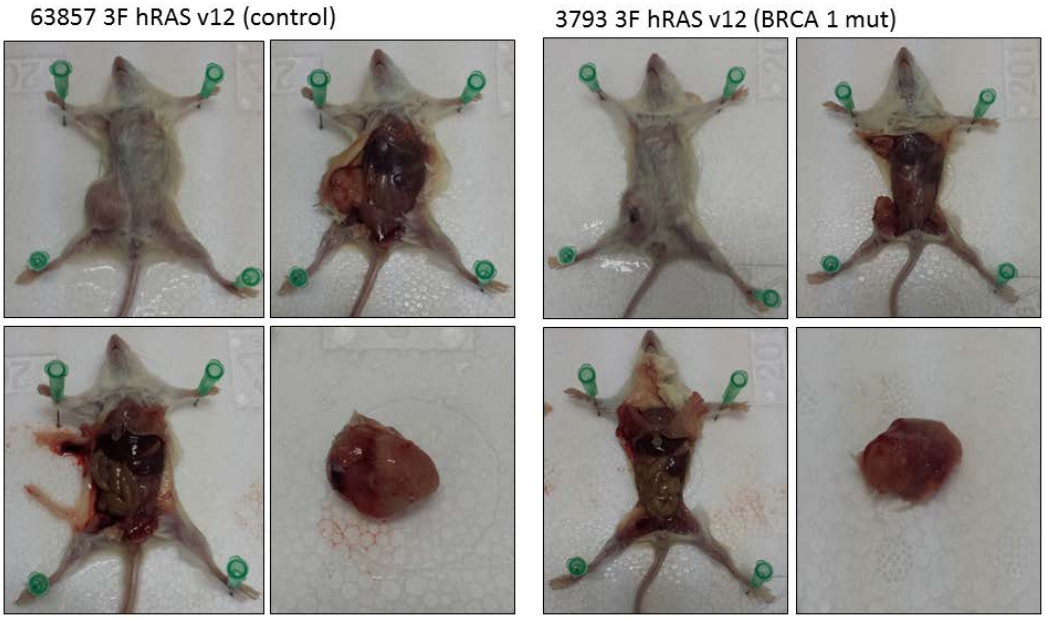


Figure **4A**: Mammary fat pad injections for 63857 3F hRAS v12 (left) and 3793 3F hRAS v12 (right) shows the development of tumors. Tumors were fixed in formalin and sent for histology. **B**: Intraperitoneal injections were performed on NSG mice. Arrows indicate location of tumor development. Tumor nodules were found embedded on the mesocolon and the abdominal cavity of both cases. Mice exhibited lethargy and distended abdomen.

Future directions - We will continue to monitor mice for tumor development. Furthermore, we will perform histological analyses on mouse tumor samples along with genomic analyses to answer objectives.

Summary of Task 1, Task 2 and Task 3

Task 1

1. Completed histological reviews of fallopian tubes from BRCA1/2 mutation carriers, along with matching controls and cancers.
2. Completed selection of a minimum of 32 fallopian tubes from *BRCA1* carriers, 32 from normal controls, 16 STICs, and 16 serous cancers from BRCA1 mutation carriers
3. Completed microdissection of selected tissue samples.
4. Gene expression profile of cellular samples.
    1) Completed gene expression array on ampulla versus fimbria from cryo-preserved tissue **– manuscript preparation – expected completion and submission December 2016.**
    2) Completed RNA-Sequencing on FFPE fimbria from BRCA1/2 mutation carriers and non-carriers.
    3) Analysis of expression profiles with focus on genes differentially expressed in *BRCA1* mutation carriers **Still to be done**

Task 2

1. Completed creation of: 2 histologically normal FTE (FTEb, FTEn, luteal and follicular designations) TMAs; 3 HGSC TMAs.
2. Completed validation of selected genes by quantitative RT-PCR: LKB1 (Oncogene 2016), GSTA2, CEBPD ER, PR, DKK3, SOX11, TKTL1 (manuscript preparation).
3. Completed selection of antibodies for validation of protein expression in normal FTE, STIC and HGSC: CD3, CD8, CD68 (Clinical Cancer Research 2012), p16/Rb, CCNE1, CCND1 (Modern Pathology 2014), LKB1 (Oncogene 2016), GSTA2, ME1, CK7 (In preparation), CEBPD (In preparation), Estrogen and Progesterone receptors, DKK3, SOX11, TKTL1 (In preparation).

Task 3

1. Completed isolation and propagation of primary FTE (n=15 cell) cell lines, including characterization and growth kinetics of the established cell lines.
2. Completed generation of pre-malignant (immortalized/non-tumorigenic) FTE cell lines (FTE-p53 R175H, FTE- p53 R175H +hTert, FTE-E7+hTert.
3. Definition of phenotypes associated with pre-malignant FTE-BRCA vs FTE-nonBRCA is an ongoing process that is dependent on the genes understudy (for example, immune infiltrates, CEBPD and GSTA2)
4. We chose to focus on in vitro cell culture assays, rather than CAM assays. This work is in progress and some of this work has been published (Oncogene 2015) and manuscripts are in preparation.
5. We have generated xenograft tumors in vivo. We are currently characterizing the xenograft derived-tumors and will be sending samples for genomic analyses. 3F = (p53 DN R175H, E7, hTERT). The tumors generated from transformed OSE and FTE, in NSG assay, they will undergo routinely molecular characterization – molecular pathology and genomics. We anticipate characterizing a maximum of 28 independent xenograft derived tumors. In particular, tumors are currently being screened for expression of: vimentin, p53, smooth muscle actin, CK7, PAX8, reticulin, EPCAM, CK18, calretinin, caldesmon and WT1. Molecular genomic analysis using the U1333 Affymetrix gene expression array and Affymetrix 6.0 SNP assay will be performed. The molecular profiles will be compared with existing HGSC expression profiles.

Training and professional development has the project provided?

- One lab member attended the 8th Canadian Conference on Ovarian Cancer Research held in Niagara Falls, Ontario. By attending this conference, he broadened his knowledge of ovarian cancer and the relevant research being conducted in the field.
- Lab members have attended numerous conferences and presented abstracts (please refer to conference section below for list of conferences attended). Our oral presentation, knowledge of field of study, critical thinking skills have developed as a result of these activities.

Dissemination to communities of interest

- Nothing to Report

Plans for the next reporting period to accomplish the goals

To successfully complete the project we plan on fulfilling the goals outlined in the statement of work. This will be achieved by continuing research activities for Tasks 2 and 3 which includes:

- **Finalizing and submitting prepared manuscripts**

- Analyzing data relevant to LCM FFPE project
- Validating and characterizing genes from this arm of the project.
- Monitoring mice for tumor development
- Characterizing xenograft tumors
- Build 4th HGSC TMA

Project 5

All relevant tissue sections have been sent to John Hopkins University. Completed – October 2015

4. **IMPACT:**

Our project focuses on supporting the notion that the fallopian tube and not the Ovarian Surface Epithelium is the tissue origin of High Grade Serous Carcinoma. To this end we have investigated the fallopian tube epithelium to a great extent and have identified several findings relevant to the advancement of knowledge in the field of ovarian cancer. We have identified that a difference in gene expression exists between patients with BRCA1 mutation status and a non-mutated status. We have gone further to study these differentially expressed genes that include LKB1, GSTA2 and CEBPD. Out results have demonstrated that a loss of LKB1 in conjunction with a mutated p53 gene results in a loss of apical to basal polarity and a loss of ciliated cells in fallopian tube cells suggesting that a loss of LKB1 disrupts the fallopian tube epithelium. With our microarray results we identified that a loss of LKB1 is frequently observed in tubal cancer precursor lesions suggesting that the loss of this gene may be an early event in disease progression. These results indicate that LKB1 may be a therapeutic target for disease control.

Our research has also focused on demonstrating a significant difference in the percentage of leukocytes in serous tubal intraepithelial lesions and HGSC cases compared to normal cases. Our results suggest that leukocytes may play a role in the progression or inhibition of the disease early. Additionally, these results were independent of ovarian cycle. We demonstrated no inherent difference in proliferation between normal and BRCA1/2 mutation carriers at the distal fallopian tube epithelium. However, a proliferative difference is present at later stages of disease progression when an early lesion is identifiable suggesting that molecular changes to the tubal epithelium occur later in the development the disease.

We have also demonstrated that a deregulated Rb1 pathway is correlated with clinical outcomes and may play a role in the development of early serous tumor development. Furthermore, RB1 may be a target for chemotherapeutic and novel therapeutic strategies. In addition to this gene, by comparing

Together our results along with manuscripts under preparation have broadened our understanding of ovarian cancer by providing an early events context to the disease.

- **What was the impact on other disciplines?**

Immunotherapy has emerged in recent years as a successful therapeutic modality that is capable of effecting durable long-term remissions. In particular, recent advances in understanding tumor mechanisms of immunosuppression have led to new treatments focused on immune checkpoint blockade, which have proven effective in a subset of patients with malignancies such as melanoma, lung cancer, and colorectal cancer. Individually, however, immune checkpoint inhibitors have shown activity in only a modest percentage of patients with these types of cancer. While a clinical trial to evaluate immune checkpoint inhibitor therapy in

ovarian cancer has been initiated, it has been reported that only 10-20% of patients will benefit from checkpoint blockade as a monotherapy in ovarian cancer. Accordingly, additional strategies are needed to prospectively identify the subset of patients who will most likely benefit from this approach, and to further enhance therapeutic efficacy. Understanding ovarian cancer immunobiology in relation to genetic defects in DNA repair pathways, which may generate neo-antigens that could serve as

effective targets for immunotherapy, but which may also affect infiltrating immunocytes through changes in exosome-mediated paracrine signaling, will enable us to develop methods for prospectively identifying ovarian cancer patients who will show significant responses to immunotherapy.

- **What was the impact on technology transfer?**

"Nothing to Report."

- **What was the impact on society beyond science and technology?**

"Nothing to Report."

- **CHANGES/PROBLEMS:**

"Nothing to Report."

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

"Nothing to Report."

5. **PRODUCTS:**

Sophia HL George, Ramlogan Sowamber, Omar Nelson, Anca Milea, Noor Salman Leah Dodds, Brian Slomovitz and Patricia Shaw. Fimbria and Ampulla Tubal Epithelium Have Similar Transcriptome Profiles. ***In preparation***

Sophia HL George, Ramlogan Sowamber, Anca Milea, Noor Salman and Patricia Shaw. The role of estrogen and progesterone in HGSC. ***In preparation***

Ramlogan Sowamber*, Rania Chehade, Noor Salman, Mahmoud Bitar, Patricia Shaw, Sophia HL George. Characterizing the role of CEBPD in High Grade Serous Carcinoma.* ***In Preparation***

Patricia A. Shaw and Blaise A. Clarke. Prophylactic Gynecologic Specimens from Hereditary Cancer Carriers. Surgical Pathology Clinics. June 2016 doi:10.1016/j.path.2016.02.002

Sophia HL George, Anca Milea, Ramlogan Sowamber, Alicia Tone, Rania Chehade and Patricia Shaw. Loss of LKB1 Protein Expression is Frequent in Serous Carcinoma. *Oncogene, 23 March 2015* doi: 10.1038/onc.2015.62

Sophia HL George and Patricia Shaw. BRCA and the fallopian tube epithelium. *Frontiers in Oncology, 2014 Jan 23*

- **Books or other non-periodical, one-time publications.**

Patricia Shaw, Blaise Clarke and Sophia HL George. Precancerous lesions of high-grade pelvic serous carcinoma, Precancerous lesions of the gynecologic tract: diagnostic and molecular pathology. Springer 2015

- **Other publications, conference papers, and presentations.**

Abstracts

S George, A Milea, N Salman and P Shaw. Fimbria and Ampulla Tubal Epithelium Have Similar Transcriptome Profiles. *Lab Invest. 95, 286A-286A 2015*

Sophia HL George, Anca Milea, Ramlogan Sowamber, Danielle Toccalino and Patricia Shaw. BRCA and early events in the development of serous ovarian cancer. *Current Oncology 21:e377 (2) April 2014*

Sophia HL George, Anca Milea, Ramlogan Sowamber, Danielle Toccalino and Patricia Shaw. The role of estrogen receptor signaling in serous ovarian cancer. *Cancer Research August 14, 2013 73:4765; doi:10.1158/1538-7445.AM2013-4765*

Kolin, D., George, S., Milea, A., Narod, S., Clarke, B., & Shaw, P. (2015, February). The Familial Ovarian Tumor Study: A Morphological and Immunohistochemical Review. In *LABORATORY INVESTIGATION* (Vol. 95, pp. 293A-293A). 75 VARICK ST, 9TH FLR, NEW YORK, NY 10013-1917 USA: NATURE PUBLISHING GROUP.

Peerani, R., George, S., Sowamber, R., Siu, A., Shao, T., Milea, A., ... & Shaw, P. (2015, February). Tumor Infiltrating Lymphocytes (TILs) as a Function of Histological Subtype and Genetic Background of Ovarian Epithelial Carcinomas. In *LABORATORY INVESTIGATION* (Vol. 95, pp. 301A-301A). 75 VARICK ST, 9TH FLR, NEW YORK, NY 10013-1917 USA: NATURE PUBLISHING GROUP.

Sophia HL George, Ramlogan Sowamber and Patricia Shaw. The role of BRCA and CEBPD in Serous Ovarian Cancer. Clin Cancer Res August 15 2015 (21) (16 Supplement) POSTER-BIOL-1312; **DOI:**10.1158/1557-3265.OVCASYMP14-POSTER-BIOL-1312

Presentations

2016	8th Canadian conference on ovarian cancer research (attendance)
2015	AACR Advances in Ovarian Cancer Meeting, Orlando, FL
2015	8th AACR The Science of Cancer Health Disparities, Atlanta, GA
2015	106th AACR Annual Meeting, Philadelphia, PA
2014	Masha Rivkin Ovarian Cancer Conference, Seattle, WA
2014	Dalla Lana School of Public Health Research and Practice Day presentation
2014	5th International Symposium on Hereditary Breast and Ovarian Cancer, QC

- **Website(s) or other Internet site(s)**

  Nothing to Report

- **Technologies or techniques**

  Nothing to Report

- **Inventions, patent applications, and/or licenses**
  Nothing to Report

- **Other Products**

  Nothing to Report

6. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

- **Individuals who have worked on the project**

Name:	*Patricia Shaw*
Project Role:	*Principle Investigator*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*90*
Contribution to Project:	*Dr. Shaw has conceptualized and executed the objectives of this project, wrote manuscripts and presented findings.*
Funding Support:	

Name:	*Sophia HL George*
Project Role:	*Scientific Associate*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*90*
Contribution to Project:	*Dr. George has conceptualized and performed experiments, wrote manuscripts and presented findings.*

Funding Support:	

Name:	*Ramlogan Sowamber*
Project Role:	*Research Staff*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*55*
Contribution to Project:	*Ramlogan has carried out experiments to generate results for the project.*
Funding Support:	

Name:	*Nick Chauvin*
Project Role:	*Research Staff*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*55*
Contribution to Project:	*Nick organized and helped with shipping DOD Project 5 slides.*
Funding Support:	

Name:	*Noor Salman*
Project Role:	*Research Staff*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*18*

Contribution to Project:	*Noor has carried out experiments to meet objectives of the project.*
Funding Support:	

Name:	*Zahra Maamir*
Project Role:	*Research Staff*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*18*
Contribution to Project:	*Zahra carried out experiments to meet objectives of the project, which included western blots*
Funding Support:	

Name:	*Gillian Geddie*
Project Role:	*Research Staff*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*18*
Contribution to Project:	*Gillian assisted with organize tissue, slides and blocks for numerous projects.*
Funding Support:	

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

  Dr. George was recently appointed to the position of Research Assistant Professor in the Department of Obstetrics and Gynecology at the University of Miami in Miami, Florida

- **What other organizations were involved as partners?**

  - **Organization Name: Princess Margaret Cancer Centre**
  - **Location of Organization:** *Toronto, Ontario, Canada*

- **Partner's contribution to the project** *(identify one or more)*
  - **Financial support - Nothing to report**
  - **In-kind support** - Desktop computer
  - **Facilities** – *Facilities and lab space, lab supplies*
  - **Collaboration** - Nothing to report
  - **Personnel exchanges** - Nothing to report
  - **Other.**

9. APPENDICES:

LCM tables

	# of cases	# cases with RNA extracted	# cases with RNA analyzed	#cases with sequence complete- October 2016
BRCA Luteal	12	12	12	12
BRCA Follicular	23	23	23	23
Normal Luteal	11	11	11	11
Normal Follicular	13	13	13	13
Cancers	9	9	9	9
Post-Menopausal	2	2	2	0
Total	70	65	19	68

Sample ID	Mutation Status	Cycle	Tissue Site	Qubit conc ng/ul	RNA Sequenced
FFPE 1	BRCA1 185delAG	Luteal	Fimbria	19.2	Yes
FFPE 2	BRCA1_3825del8	Follicular	Fimbria	24.4	Yes

FFPE 3	no mutation	Follicular	Fimbria	32	Yes
FFPE 4	BRCA1 1806C-T	Follicular	Fimbria	57	Yes
FFPE 5	no mutation	Luteal	Fimbria	4.4	Yes
FFPE 6	BRCA1	Luteal	Fimbria	64.6	Yes
FFPE 7	BRCA1	Follicular	Fimbria	8.78	Yes
FFPE 8	BRCA1 unknown mutation	Follicular	Fimbria	12.5	Yes
FFPE 9	BRCA1-1294del40 2434T-C variant	Follicular	Fimbria	64.2	Yes
FFPE 10	BRCA1 185delAG	Luteal	Fimbria	29.8	Yes
FFPE 11	BRCA1	HGSC	Tumor	71.6	Yes
FFPE 12	BRCA1	HGSC	Tumor	20.6	Yes
FFPE 13	BRCA1	HGSC	Tumor	94.8	Yes
FFPE 14	BRCA1	HGSC	Tumor	54.4	Yes
FFPE 15	BRCA1	HGSC	Tumor	102	Yes
FFPE 16	BRCA1	HGSC	Tumor	20.8	Yes
FFPE 17	BRCA1	HGSC	Tumor	39.8	Yes
FFPE 18	BRCA1	HGSC	Tumor	60.6	Yes
FFPE 19	BRCA1	HGSC	Tumor	84.2	Yes
FFPE20	no mutation	Follicular	Fimbria	13.1	Yes
FFPE21	BRCA1 185delAG	Luteal	Fimbria	20.6	Yes
FFPE22	BRCA1 4239 delAG	Follicular	Fimbria	10.9	Yes
FFPE23	no mutation	Follicular	Fimbria	21.8	Yes
FFPE24	BRCA1_1479delAG	Follicular	Fimbria	19.5	Yes
FFPE25	BRCA2	Follicular	Fimbria	11	Yes
FFPE26	BRCA2_6174delT	post-menopausal	Fimbria	6.9	Yes
FFPE27	BRCA1 5382insC	Follicular	Fimbria	4.2	Yes
FFPE28	BRCA1-185delAG	Luteal	Fimbria	4.2	Yes
FFPE30	FOC/BRCA2	Luteal	Fimbria	12.5	Yes
FFPE31	BRCA1_5382insC	Follicular	Fimbria	9.08	Yes

FFPE32	no mutation	Follicular	Fimbria	12.2	Yes
FFPE33	BRCA1 3533 INSG mutation	Follicular	Fimbria	8.74	Yes
FFPE34	BRCA1 185delAG	Luteal	Fimbria	16.1	Yes
FFPE35	BRCA1	Follicular	Fimbria	17.2	Yes
FFPE36	BRCA1 185delAG	Follicular	Fimbria	20.8	Yes
FFPE37	BRCA1 g. 56490A>G	Follicular	Fimbria	15.3	Yes
FFPE38	BRCA1 185delAG	Follicular	Fimbria	27.2	Yes
FFPE39	no mutation	Luteal	Fimbria	12.5	Yes
FFPE40	BRCA1 unknown mutation	Luteal	Fimbria	12.8	Yes
FFPE41	BRCA1 917delTT	Follicular	Fimbria	29.6	Yes
FFPE42	BRCA1 185delAG	Luteal	Fimbria	30.8	Yes
FFPE43	no mutation	Follicular	Fimbria	23.6	Yes
FFPE44	no mutation	Follicular	Fimbria	52.2	Yes
FFPE 45	BRCA1_101delGT	Follicular	Fimbria	9.12	Yes
FFPE 46	BRCA1_4603G-T	peri/post menopausal	Fimbria	26	Yes
FFPE 47	BRCA1_129del40	Luteal	Fimbria	25	Yes
FFPE 48	BRCA1_3450delCAAG	Follicular	Fimbria	35	Yes
FFPE 49	BRCA2	Follicular	Fimbria	40.8	Yes
FFPE 50	no mutation	Follicular	Fimbria	10.2	Yes
FFPE 51	no mutation	Follicular	Fimbria	21.2	Yes
FFPE 52	no mutation	Follicular	Fimbria	99.6	Yes
FFPE 53	no mutation	Follicular	Fimbria	29.2	Yes
FFPE 54	no mutation	Follicular	Fimbria	80.6	Yes
FFPE 55	no mutation	Luteal	Fimbria	*	Yes
FFPE 56	no mutation	Luteal	Fimbria	*	Yes
FFPE 57	no mutation	Luteal	Fimbria	9.8	Yes

FFPE 58	no mutation	Luteal	Fimbria	6.52	Yes
FFPE 59	no mutation	Luteal	Fimbria	*	Yes
FFPE 60	no mutation	Luteal	Fimbria	*	Yes
FFPE 61	no mutation	Follicular	Fimbria	*	Yes
FFPE 62	BRCA1	Follicular	Fimbria	16.6	Yes
FFPE 63	BRCA1	Luteal	Fimbria	86.6	Yes
FFPE 64	BRCA1	Follicular	Fimbria	15.4	Yes
FFPE 65	BRCA1	Follicular	Fimbria	11.6	Yes
FFPE 66	BRCA1 185delAG	Luteal	Fimbria	*	Yes
FFPE 67	no mutation	Follicular	Fimbria	*	Yes
FFPE 69	BRCA2_5783delT	Follicular	Fimbria	14	Yes
FFPE 70	no mutation	Luteal	Fimbria	*	Yes
FFPE 71	no mutation	Luteal	Fimbria	*	Yes
FFPE 72	no mutation	Luteal	Fimbria	*	Yes
	* Awaiting values from sequencing department				

Table2: The above cases are for the LCM FFPE project. All cases have had RNA quantified and RNA sequenced.

Cell Line	**BRCA mutation status**
iOSE 120 SV40, hTERT hRAS v12	control
iOSE 120 SV40 hTERT c-MYC	control
iOSE 267F SV40 hTERT hRAS v12	BRCA1 mut
iOSE 267F SV40 hTERT c-MYC	BRCA1 mut
iOSE 523 SV40 hTERT c-MYC	control
iOSE 592F SV40 hTERT c-MYC	BRCA1 mut
iOSE 523 SV40 hTERT hRAS v12	control
iOSE 592F SV40 hTERT hRAS v12	BRCA1 mut
FTE 3437 SV40 hTERT hRAS v12	control, 85yrs

FTE 3437 SV40 hTERT c-MYC	control, 85yrs
FTE 3313 SV40 hTERT c-MYC	BRCA2 mut, post
FTE 3798 SV40 hTERT hRAS v12	BRCA1 mut, 45yr
FTE 3798 SV40 hTERT c-MYC	BRCA1 mut, 45yr
FTE 3313 SV40 hTERT hRAS v12	BRCA2 mut, post
FTE 3619 SV40 hTERT hRAS v12	control, 48yrs
FTE 3793 3F CCNE1 c-MYC	BRCA1 mut, 47yrs
FTE 3793 3F PIK3CA-H1047R c-MYC	BRCA1 mut, 47yrs
FTE 3319 3F PIK3CA-H1047R CCNE1	control, 56yrs
FTE 3319 3F PIK3CA-H1047R hRAS v12	control, 56yrs
FTE 3793 3F hRAS v12	BRCA1 mut, 47yrs
FTE 3313 3F c-MYC	BRCA2 mut, post
FTE 3313 3F hRAS v12	BRCA2 mut, post
FTE 3793 3F PIK3CA-H1047R CCNE1	BRCA1 mut, 47yrs
FTE 3319 3F PIK3CA-H1047R c-MYC	control, 56yrs
FTE 3619 SV40 hTERT c-MYC	control, 56yrs
FTE 3793 3F PIK3CA-H1047R hRAS v12	BRCA1 mut, 47yrs
FTE 63857 3F PIK3CA-H1047R	control, 29yrs
FTE 63857 3F CCNE1	control, 29yrs
FTE 63857 3F hRAS v12	control, 29yrs
FTE 3313 3F PIK3CA-H1047R hRASv12	BRCA2 mut, post
FTE 63857 3F PIK3CA- H1047R hRASv12	control, 29yrs
FTE 3793 3F PIK3CA - H1047R	BRCA1 mut, 47yrs
FTE 3313 3F PIK3CA-H1047R	BRCA2 mut, post
FTE 3319 3F hRASv12	control, 56yrs
FTE 3319 3F PIK3CC-H1047R	control, 56yrs
FTE 3437 SV40 hTERT hRas v12 -IP	control, 85yrs
FTE 63857 3F hRasv12 -IP	control, 29yrs
iOSE 523 SV40 hTERT hRasv12 -IP	control

iOSE 3793 hRas v12 -IP	BRCA1 mut, 47yrs
iOSE 267F SV40 hTERT hRas v12 -IP	BRCA1 mut
FTE 3798 SV40 hTERT hRas v12 -IP	BRCA1 mut, 45yr
3F - (p53 DN R175H, E7, hTERT)	
CCNE1 - Cyclin E	
IP- intraperitoneal injections	

Table 3: Summary of injections to date. Intraperitoneal injections were performed in addition to mammary fat pad injections in NSG mice.

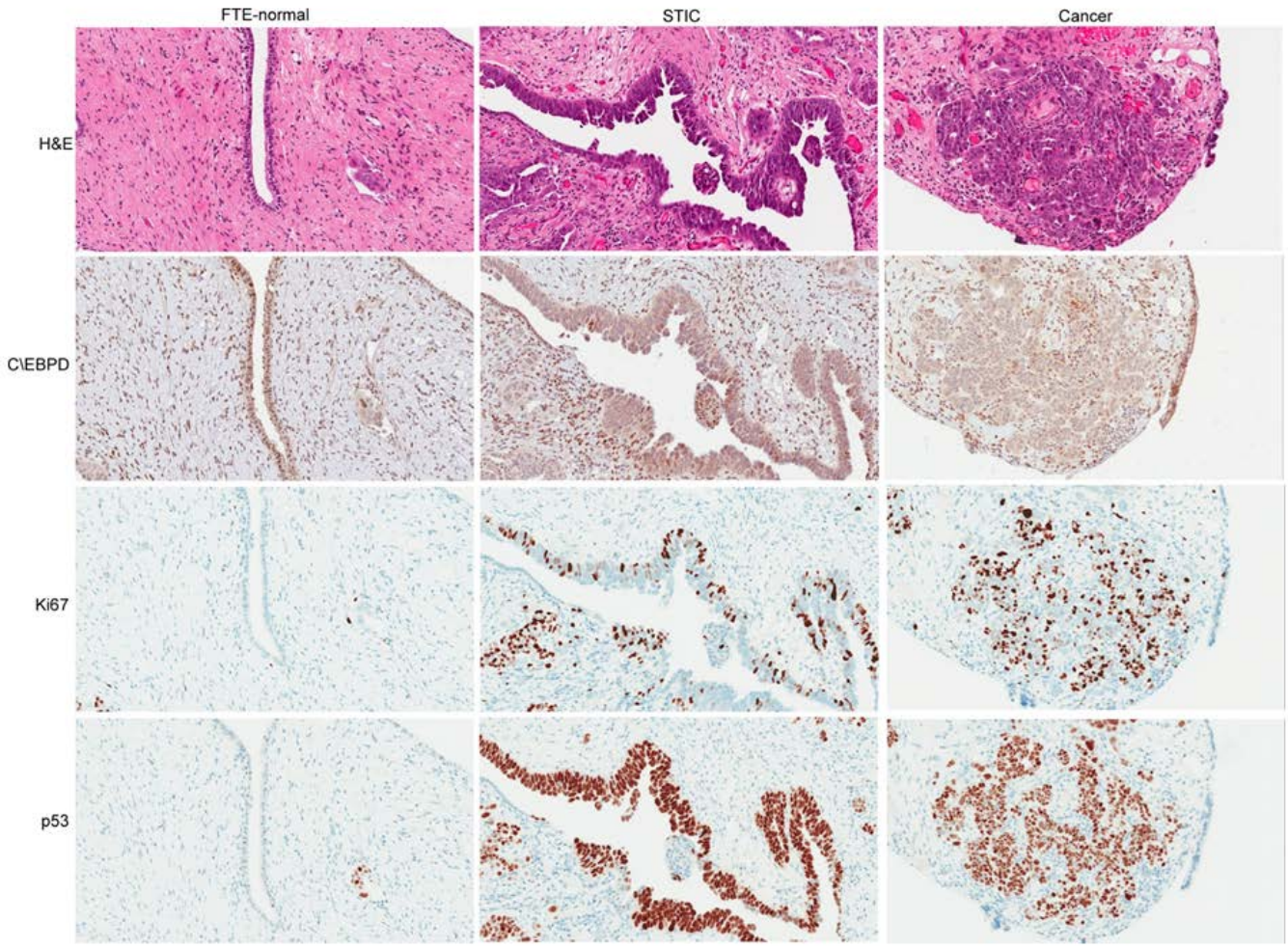


Figure 1: CEBPD is high in normal tissue and low in HGSC. This indicates that CEBPD expression may be lost as tumor development occurs.

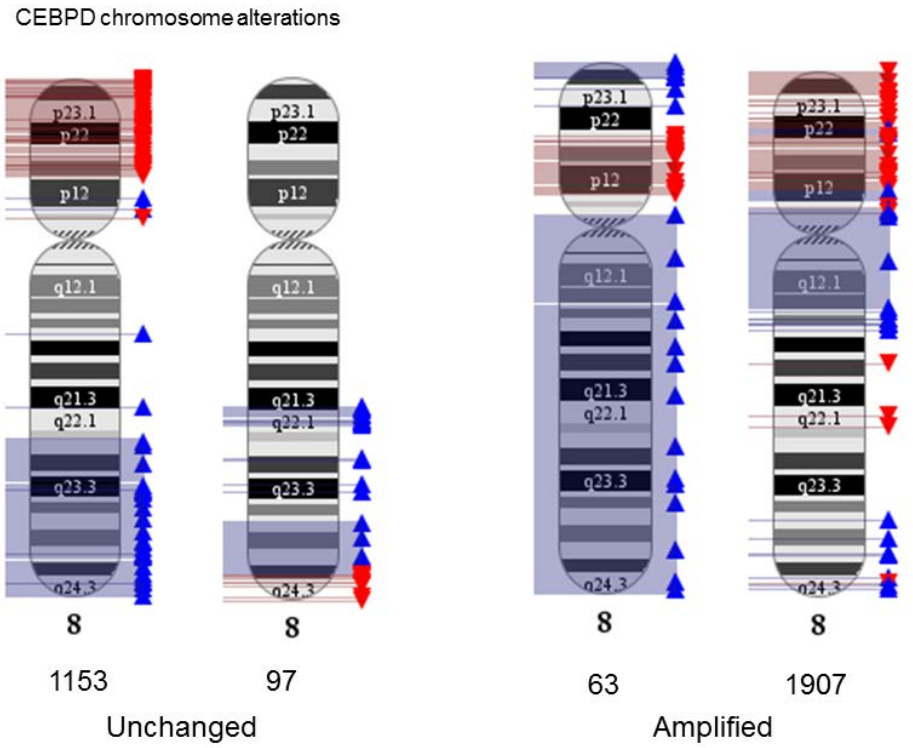


Figure 2: 75 HGSC were analyzed for copy number alterations. CEBPD was altered on Chromosome 8q11.21. There were a total of 15 aberrations with 13 amplifications and 2 deletions. 60 cases were unchanged.

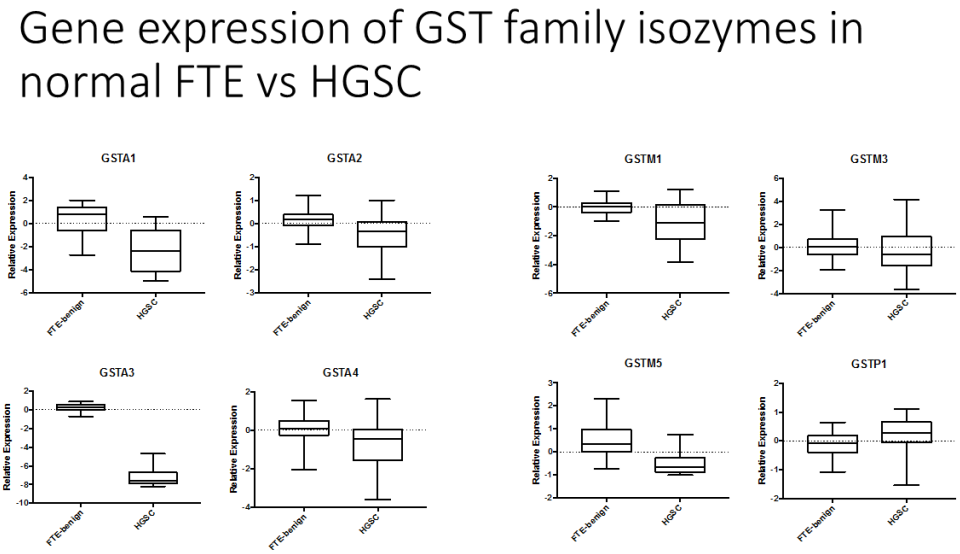


Figure 3: Control blot demonstrating epithelial nature of cell lines utilized for experiments.

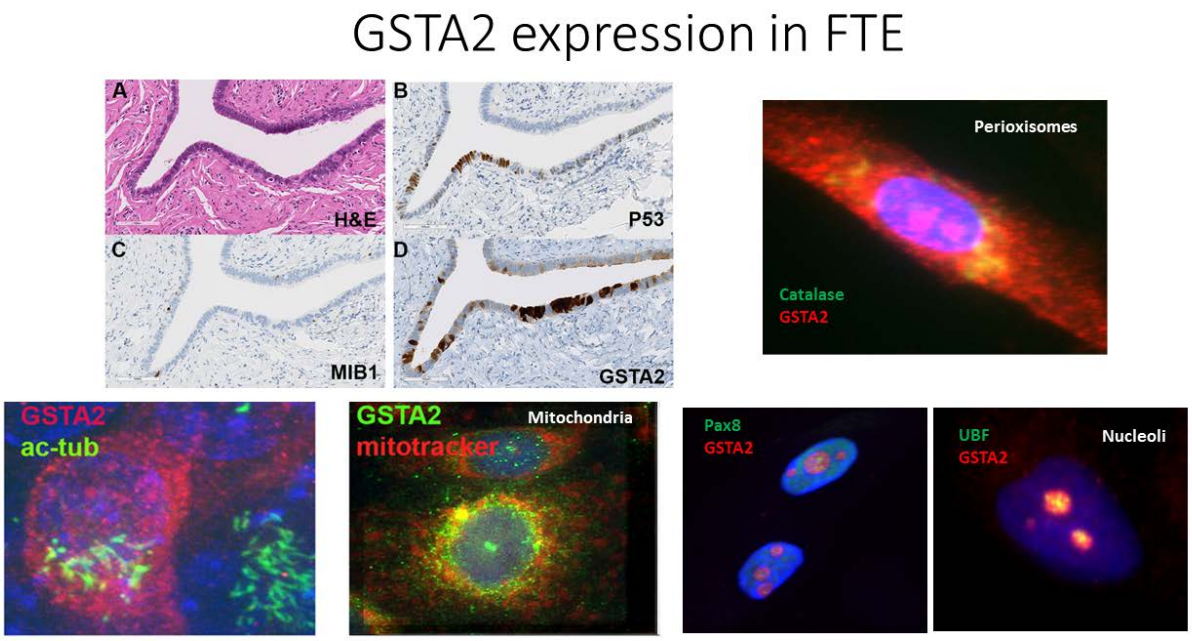
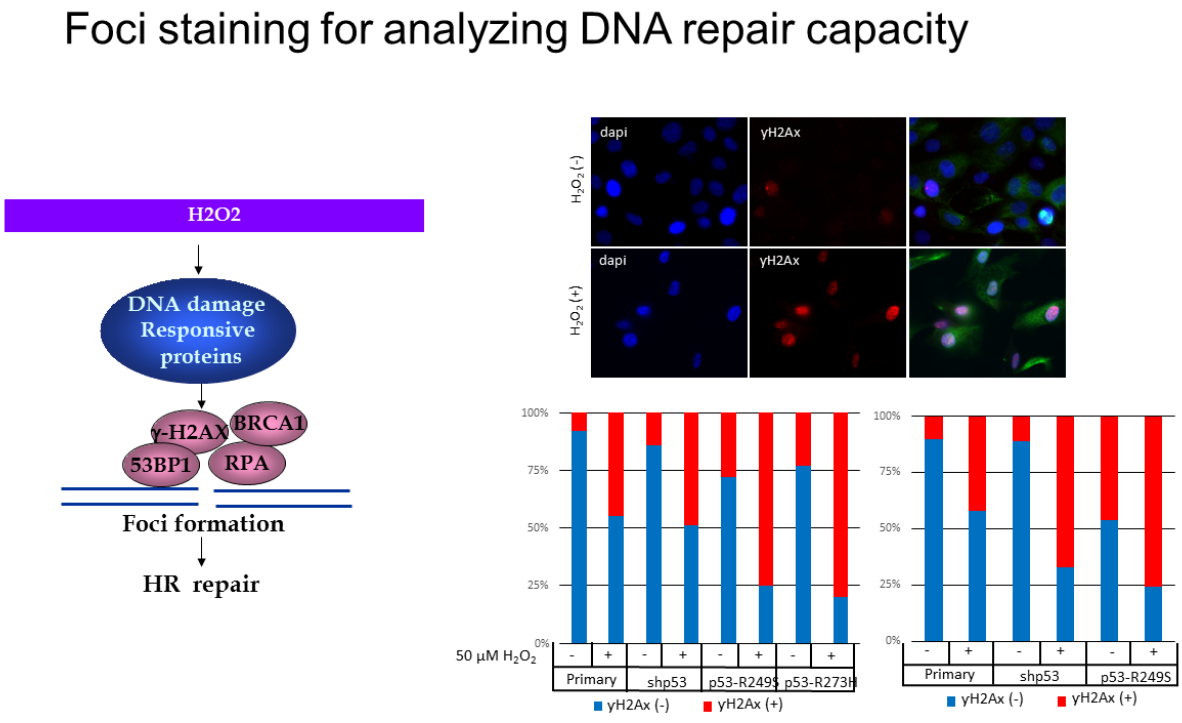


Figure 4: Glutathione-S-transferase isozymes are differentially expressed between normal FTE and HGSC. In normal FTE cells, GSTA2 is expressed in mitochondria, perioxisomes and nucleoli. These enzymes may indirectly regulate RNA biogenesis.



Attenuation of DNA damage response in FTE

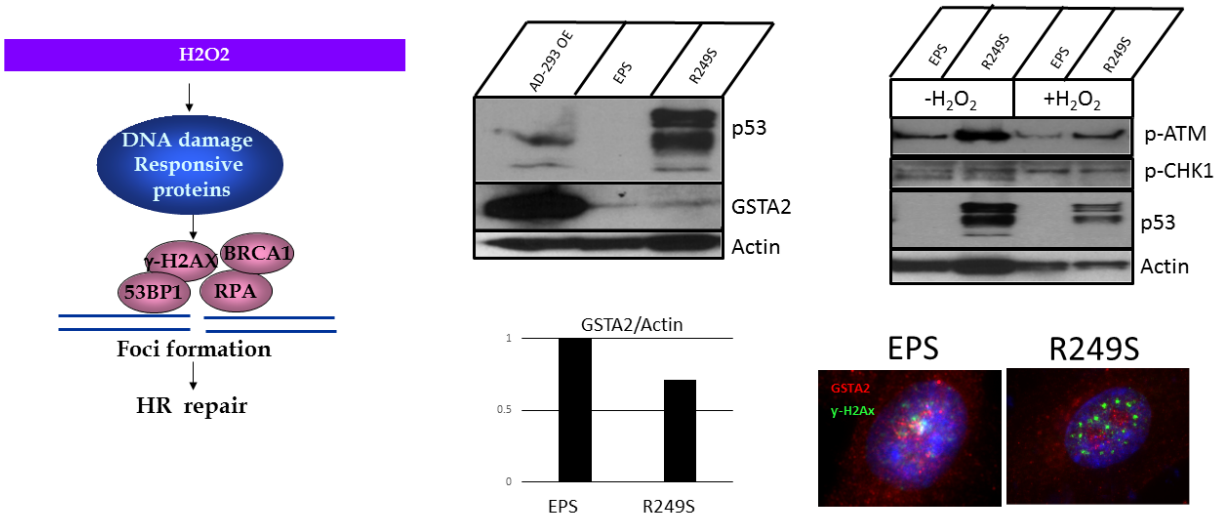


Figure 5: DNA-damage induced gamma H2AX foci does not stimulate GSTA2 expression in FTE cells with a dominant negative p53.

Reconstitution of GSTA2 in ovarian cancer cells does is no sufficient to abrogate DNA damage

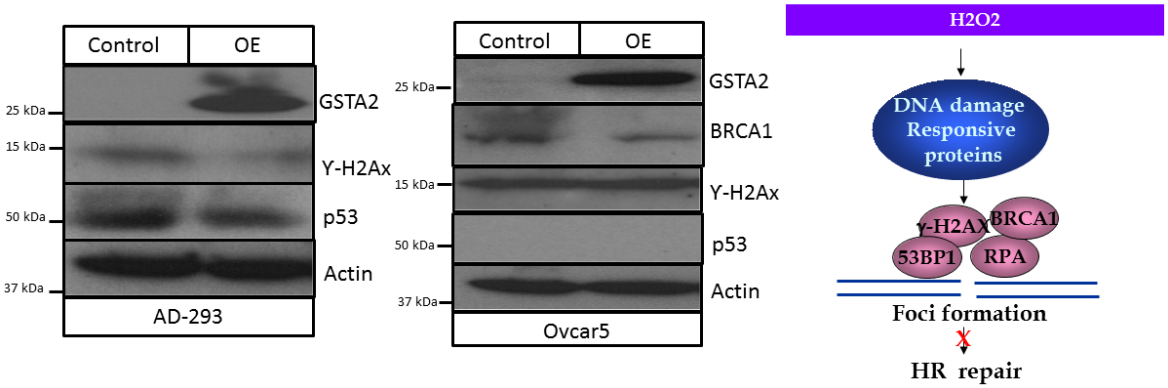


Figure 6: Over-expression of GSTA2 in ovarian cancer cell lines (OVCAR5) does not rescue DNA damage response (measured by gamma H2AX expression) .

Project 4. Locate and characterize precursor lesions of "ovarian" cancer in a mouse model and explore the role of ovulation and changes in the microenvironment of the ovary and tube in "ovarian" carcinogenesis using human tubal xenografts in nude mice.
Research site: Johns Hopkins University
Project Leader: Tian-Li Wang
Co-investigators: Ie-Ming Shih (JHU)

Section II. Progress to Date

Task 1. Locate the anatomic site and characterize the precursor lesions of "ovarian" cancer in the $TP53^{-/-}/Rb^{-/-}$ mouse model.

Task 1a. Mouse breeding and genotyping (1-18 months)
Progress: This task has been finished.

Task 1b. TP53/Rb KO mice study to locate the anatomic site of precursor lesions (1-15 months).
Progress: As discussed in the previous progress reports, this task has been modified and the final result has been published: J Pathol. 2014 Jul;233(3):228-37. This paper is highly cited as there are more than 33 citations in two years (based on Google Scholar).

Task 1c. Characterize the mouse precursor lesions using a variety of proposed methods (10-33 months).
Progress: This task has been completed and the data has been published and presented in the last progress report and will not be re-iterated in the current report. *J Pathol, 2014. PMID:24652535*

Task 2. Assess the biological effects of ovarian follicular fluid on human fallopian tube epithelium (FTE) and ovarian surface epithelium in a xenograft model.

Task 2a. Establish the human fallopian tube xenograft model (18-30 months).
Progress: We have finished exploring this model for the purpose of ovarian cancer research in mice and the results were shown in the previous progress report (with a figure illustration). So, the results will not be reiterated herein.

Task 2b. Collect human follicular fluid and primary characterization of the fluid (15-25 months).
Progress: This task is in progress. We will finish this task in the period of no cost extension.

Task 2c. Assess the biological effects of ovarian follicular fluids on FTE and OSE in a xenograft model (30-48 months).
Progress: As described in the previous reports, we have collected human follicular fluids and human tubal fluids and tested their DNA damaging effects on FT cultures. As described in the last progress report, we have performed the comet assay and gH2A staining and Western blot analyses to quantitate the damages in double strand DNA. Application of follicular fluid to the mouse xenograft model (Task a) appears challenging as the fluid may not penetrate into the grafted fallopian tube epithelium which is embedded by fibrotic tissues. Alternatively, we are considering an alternative study to expose the fresh human fallopian tube to the fluid and analyze DNA damage levels after exposure at different time points.

Task 3. Determine whether oral contraceptives (OCPs) and NSAIDs reduce the morphologic and molecular changes that are associated with early "ovarian" carcinogenesis.

Task 3a. To assess whether OCPs decrease the frequency of precursor lesions and/or delay tumor development (24-48 months)
Progress: We have finished this task and found that there was no difference in the experimental and control groups after exposure of OCPs.

Task 3b. To determine the effects of aspirin on reducing oxidative stress-induced molecular changes on human fallopian tube and/or on OSE (24-60 months).
Progress: This task has been modified as described in the previous progress reports. The results have been published in Clin Cancer Res 2015 Oct 15;21(20):4652-62. We demonstrate that lovastatin significantly reduced the development of STICs in mogp-TAg mice and inhibited ovarian tumor growth in the mouse xenograft model. Knockdown of prenylation enzymes in the mevalonate pathway recapitulated the lovastatin-induced antiproliferative phenotype. Transcriptome analysis indicated that lovastatin affected the expression of genes associated with DNA replication, Rho/PLC signaling, glycolysis, and cholesterol biosynthesis pathways, suggesting that statins have pleiotropic effects on tumor cells. The above results suggest that repurposing statin drugs for ovarian cancer may provide a promising strategy to prevent and manage this devastating disease.

Task 3c. Data analysis and preparation for publication (24-60 months).
Progress: Our next publication target will focus on reporting that biosphosphonate can reduce the STIC and HGSC formation in the OVGP1-statin mouse model.

Section III. Problem Areas for Project 4

There are no major problem areas noted for Project 4 as alternative approaches are proposed to address the same questions as originally proposed.

Section IV. Future Work in Project 4

Project 4 has made substantial progress toward completing the proposed main tasks. However, Task 2 needs to be finished in order to meet the overall objective of the Consortium program. As originally proposed, Task 2 will assess the biological effects of ovarian follicular fluid on human fallopian tube epithelium (FTE) and ovarian surface epithelium in a xenograft model (Task 2a), collect human follicular fluid and primary characterization of the fluid (Task 2b) and assess the biological effects of ovarian follicular fluids on FTE and OSE in a xenograft model (Task 2c). During the no cost extension period, we will focus on transplanting human fallopian tube segments directly to the mouse peritoneal wall. This experiment was delayed because till now we have accumulated sufficient knowledge and experience in establishing this novel surgical model. We will also share with the science community about our protocol after publication. In the past years, we also learn that the follicular fluids need to as fresh as possible for the proposed experiments in order to have a reproducible result. Therefore, we will schedule with the Laboratory Director of the infertility clinic at the Green Spring Station (part of Johns Hopkins Medicine) to personally pick up the fluid by the Project leader in the early mornings for experiments conducted on the same day. Thus, we are confident by now that we are able to move ahead to address the roles of follicular fluids in generating tubal precursor lesions. Also, for the Task 3 which is to determine whether oral contraceptives (OCPs) and NSAIDs reduce the morphologic and molecular changes that are associated with early "ovarian" carcinogenesis, we will finish up this task by concluding the drugs that offer the best

chemopreventive effects. Since mouse study almost always takes much time to finish, the no cost extension will be necessary for completing this task and tightening the loose ends for wrapping up papers for publication. The results from this no cost extension will be critical to demonstrate if there is a possibility that we can prevent ovarian cancer in high-risk women using commonly prescribed medications, and if so, the impact on women health would be tremendous.

Project 5: Epidemiologic and molecular characterization of putative precursor lesions in the ovary and fallopian tube of unaffected high-risk women.

Project 5: Determine the molecular and epidemiologic profile of putative precursor lesions in the fallopian tubes and ovaries from women at high-risk for ovarian cancer. In addition, Project 5 will determine if these biomarkers and associated precursor lesions are modifiable by oral contraceptives (OCPs) or anti-inflammatory agents, as OCPs in particular are known to prevent ovarian cancer and impact survival.
Research Site: Johns Hopkins University
Principal Investigator: Kala Visvanathan, MD, MHS
Collaborating Sites: University of Toronto (Steven Narod, MD and Patricia Shaw, MD: Co-investigators), Memorial Sloan Kettering Cancer Center (Douglas Levine, MD and Robert Soslow, MD: Co-investigator), Yale University (Vinita Parkash, MD and Ellen Matloff, MSc, MS, CGC: Co-Investigators)

Section II. Progress to Date:
Our efforts have been focused on the completion of all Project 5 aims. As stated in prior progress reports there were modifications to our original aims due to feasibility that included expanding Aim 1 and discontinuing Aim 3. We remain confident that our study outcomes will be an important contribution to the understanding of the etiology of STICs, their role in the development of ovarian cancer, and be informative in future prevention strategies.

Aim 1: This aim was expanded and divided into three sub-aims: Aim 1a is a pilot study to evaluate prevalence of STICs, p53 signatures and STILs; Aim 1b involved the creation of a large multicenter dataset to evaluate risk factors for STICs, STILS and p53signatures; and Aim 1c involved the creation of a large multicenter dataset to evaluate risk factors for ovarian cysts.

Aim 1a: Pilot Study to evaluate the prevalence of STICs, p53 signature and STILs.
We have completed Aims 1a, 1b and 1c, which involved extensive tissue sampling, immunohistochemical staining, pathological, slide reviews (2,880 slides), and entering the results for all cases (114 cases) into a database. We are currently writing up these novel results (see tables below) and will submit soon. See table 1 for the prevalence

Aim 1b & 1c: Pathological and Epidemiological data from the larger prospective/retrospective Cohort
For this aim eligible participants were identified from 2 prospective cohorts of high-risk women, Hopkins and Toronto and from MSKCC clinical database. Epidemiological and clinical data on 400 individuals was collected, cleaned and reviewed. Pathology reports were also collected.
See table 1 for the prevalence's of the lesion from both Aim 1 a and Aim 1 b. Of note is that the numbers are very similar despite the two different approaches and much smaller than earlier studies. Also it suggests we need to understand what makes a p53 signature become a STIC given its higher prevalence. Table 2 reports on selected characteristics that were significantly different in women with STICs compared to controls are and table 3 reports on differences between women with p53 signatures compared to controls. Of note positive associations were observed for similar characteristics in women with both STICs and p53 supporting the fact that p53 may be an earlier precursor for STIC. We are planning to submit this data as an abstract to the AACR Annual Meeting and the manuscript is in preparation. These results are an important contribution to our understanding of the etiology of STICs.

Table 1 Prevalence of STICS in both studies.

	Larger Study		**Pilot Study**	
STIC only	12/400	3%	4/105	3.80%
STIC + Cancer	2/400	0.50%	0	0%
Cancer only	4/400	1%	0	0%
STIL	5/400	1.25%	2/105	1.90%
p53 signature	26/400	6.50%	8/105	7.60%

Table 2: Characteristics of women with STIC and/or cancer vs. those without STIC and cancer

	Non STIC/Cancer (n=382), n (%)	**STIC/Cancer (n=18), n (%)**	**P-value***
Year of Oophorectomy	2007 (3.0)	2005 (3.4)	0.004
Age at Oophorectomy	48.1 (8.3)	52.9 (10.7)	0.019
BRCA mutation status			0.78
BRCA 1 Positive	179 (46.9)	9 (50.0)	
BRCA 2 Positive	169 (44.2)	9 (50.0)	
VUS	9 (2.4)	0 (0.0)	
BRCA 1/2 Neg	10 (2.6)	0 (0.0)	
Missing	15 (3.9)	0 (0.0)	
Body Mass Index (kg/h^2)			0.092
<25	164 (42.9)	5 (27.8)	

25-30	82 (21.5)	5 (27.8)	
>=30	66 (17.3)	1 (5.6)	
Missing	70 (18.3)	7 (38.9)	
Infertility			0.042
Never	173 (45.3)	3 (16.7)	
Ever	26 (6.8)	1 (5.6)	
Missing	183 (47.9)	14 (77.8)	
Hysterectomy			0.08
No	355 (92.9)	17 (94.4)	
Yes	24 (6.3)	0 (0.0)	
Missing	3 (0.8)	1 (5.6)	
Menopausal Status			0.35
Pre-Menopausal	231 (60.5)	8 (44.4)	
Post-Menopausal	148 (38.7)	10 (55.6)	
Missing	3 (0.8)	0 (0.0)	
Race			0.003
White	350 (91.6)	14 (77.8)	
Non-White	31 (8.1)	3 (16.7)	
Missing	1 (0.3)	1 (5.6)	
Statins			0.042
No	362 (94.8)	15 (83.3)	
Yes	20 (5.2)	3 (16.7)	
Fibroids			0.041
No	329 (86.1)	17 (94.4)	
Yes	50 (13.1)	0 (0.0)	

Missing	3 (0.8)	1 (5.6)	
*t-test used for continuous variables, fisher's exact test used for categorical variables			

Table 3: Characteristics of women with a p53 signature vs. those without a p53 signature

	Non-p53 sig (n=252), n (%)	p53 sig (n=14), n (%)	P-value*
Year of Oophorectomy	2006 (3.11)	2004 (2.72)	0.007
Age at Oophorectomy	47.6 (7.97)	51.5 (8.55)	0.07
BRCA mutation status			0.78
BRCA 1 Positive	118 (46.8)	7 (50.0)	
BRCA 2 Positive	106 (42.1)	7 (50.0)	
VUS/Other	7 (2.8)	0 (0.0)	
BRCA 1/2 Neg	9 (3.6)	0 (0.0)	
Missing	12 (4.8)	0 (0.0)	
Body Mass Index (kg/h^2)			0.26
<25	95 (37.7)	3 (21.4)	
25-30	46 (18.3)	3 (21.4)	
>=30	41 (16.3)	1 (7.1)	
Missing	70 (27.8)	7 (50.0)	
Inferility			0.075
Never	52 (20.6)	0 (0.0)	
Ever	17 (6.8)	0 (0.0)	
Missing	183 (72.6)	14 (100.0)	
Hysterectomy			0.12
No	229 (90.9)	13 (92.9)	
Yes	20 (7.9)	0 (0.0)	
Missing	3 (1.2)	1 (7.1)	

Menopausal Status			0.43
Pre-Menopausal	148 (58.7)	6 (42.9)	
Post-Menopausal	101 (40.1)	8 (57.1)	
Missing	3 (1.2)	0 (0.0)	
Race			
White	225 (89.3)	10 (71.4)	<0.001
Non-White	27 (10.7)	3 (21.4)	
Missing	0 (0.0)	1 (7.1)	
Statins			0.033
No	244 (96.8)	12 (85.7)	
Yes	8 (3.2)	2 (14.3)	
Fibroids			0.097
No	222 (88.1)	13 (92.9)	
Yes	27 (10.7)	0 (0.0)	
Missing	3 (1.2)	1 (7.1)	
*t-test used for continuous variables, fisher's exact test used for categorical variables			

All data analysis and tables have been completed and a manuscript is near completion for submission. We believe this may be the second largest study to be published and the first outside a clinical study.

<u>Aim 2:</u> Molecular and Epidemiological Characterization of STICs and p53 signatures

This aim was to be completed based on STIC and p53 signature samples collected in Aim 1 but given the low prevalence of STICs in Aim 1a and b (initial publications from small studies had reported higher numbers) this was not feasible. Therefore, we reached out to an additional 20 sites for additional STICs and have formalized collaboration with 5 sites so that we will be able to carry out our original Aim 2 as proposed. The goal is to evaluate a panel of markers in 50 p53/STIL cases, 50 STICs, 50 adjacent normal tissue and 50 matched controls. Each control will be carefully matched on age (+/- 2 years), date of procedure (+/- 2 years) to the case and study site. Aim 2 has required a significant amount of time and effort to identify and facilitate collaboration with multiple sites. We now have in place fully executed MTAs and have obtained tissue and de-identified clinical information from the majority of them. Once we have all the STICs and controls they will all undergo path review at Johns Hopkins and we will prioritize the markers to be evaluated. The potential list includes

γH2AX, pCHK2, 8-OHdG, telomere length, CD45+ lymphocytes, Ki-67 index, protein expression of p53, p21, cyclin E1, Rsf-1, fatty acid synthase, laminin C1 and progesterone receptor A and B.

We feel strongly that the results from Aim 2 will be a unique contribution to the field as it will provide important information about the etiology of STICs and will also help guide future preventative strategies. As originally proposed the selection of some of the markers will be guided by results from consortium Projects 1 and 3.

Specific Tasks

Task 1. Obtain approval for the addition of questions for prospective collection from all site IRBs (Aim 1) and also for the transfer of existing epidemiological and clinical data to JHH de-identified for the retrospective study (Aim 1 and 2). Obtain approval from USAMRAA for human subject's research for entire protocol (Months 1-6).

Progress: This task was completed. We continue to maintain current IRB approvals at each institutional site for subsequent studies.

Task 2. Identification of study population for retrospective study based on eligibility criteria from all sites (Months 1-6).

Progress: This task was completed. All epidemiological data and pathology reports on 400 cases have been identified, collected, entered, cleaned and analysis and manuscript preparation is in process.

Task 3. Comprehensive pathology review of samples. A comprehensive master dataset that includes epidemiological and pathological data from approximately 550 women to be used for cross-sectional and case control study designs will be created (Months 6-18).

Progress: Task completed. We compiled an epidemiological and pathological database of 400 high-risk women due to the low prevalence of STICs we decided not add further to this database.

Task 4. Analyses for Aims 1a and b will be completed to determine the prevalence, location and frequency of the specific lesions in the FTEs (STICs, STILs, and p53 signatures) and OSE, as well as CICs in the ovaries, overall and by BRCA mutation status. Correlations between each of the different types of lesions will be determined. Manuscript preparation Aims 1a and b (Months 6-18).

Progress: Task completed. We have examined prevalence looking at detailed pathological and molecular evaluation of 114 STIC cases in which the top and bottom of each block was samples as well as reported prevalence of 400 high-risk women who underwent prophylactic oophorecotomy. We are finalizing the manuscripts now and will also submit an abstract to AACR.

Task 5. Analyses for Aim 1c the association between exposures (both risk and protective factors for ovarian cancer) and each type of prevalent lesion will be examined through cross-sectional studies. Manuscript preparation for Aim 1c (Months 12-24).

Progress: This manuscript reporting on the 400 cases is being finalized.

Task 6. As described above, merging of epidemiological and pathological data for aims 1 and 2 will be performed and a comprehensive master dataset to be used from which matched case-control studies will be identified for Aim 2 (Months 6-18).

Progress: This task has been completed. Given the low prevalence of STICs among 400 cases we have reached out to other sites to collect 50 STICs and 50 matched control sets.

Task 7. Case-control sets for molecular analyses of the FTE and ovary samples from Aim 1 will be used to assess a panel of markers. Cases will be defined by the lesion/region of interest (i.e. STICs, STILs, p53 signatures, CICs, and/or morphological changes in OSE). Controls will vary depending on the analysis: marker expression within a specific lesion will be compared to (1) adjacent normal tissue from the same case and (2) normal tissue from women with no identifiable lesion. For the latter comparison, 2 controls will be matched to each case from the same research site, within +/- 2 years of age at the time of surgery. To conserve this valuable tissue, and maximize efficiency, the same controls will be reused for subsequent case-control
analyses where possible (Months 18-24).

Progress: We expanded the current study to include all STICs irrespective of high-risk status with a goal of 50 STIC, 50 p53 signatures cases and matched controls (described above). After contacting over 20 external sites we were able to reach our target goal. Outside collaborating sites include Cedars-Sinai, UCLA, Northwestern, Cleveland Clinic, Sibley Hospital and University of Pittsburgh.

Task 8. Molecular analyses to determine molecular profile of each lesion type will be performed on case control sets. The resulting laboratory results will be merged with existing epidemiological data and analyses will be performed (Months 24-48).

Progress: We experienced a slight delay in starting the molecular analysis as it took more time and effort than expected to collect our 50 STIC and 50 p53 case-control sets. We are moving ahead and do not anticipate any further issues with completion of the molecular analyses.

Task 9. Statistical analyses of case-control sets will be completed for each lesion type and panel of markers. Multiple manuscripts will be generated from Aim 2 based on the different lesions and also markers. These will be prepared and submitted (Months 24-48).

Progress: This will be done during the NCE period in 2017.

Task 10. High-risk women considering BSO in the next 2 years, and meeting the same eligibility criteria used in Aims 1 and 2, will be prospectively enrolled at each site. Information on NSAIDs, OCP and Vitamin E use as well as other ovarian cancer risk/protective factors will be collected through questionnaires completed within 2 years of surgery (N ~ 300-400) (Months 6-48).

Progress: Status remains same as last report. Given the low prevalence of STICs we will not proceed but have directed our focus this period of identifying unique case-control set from various institutions.

Task 11. Ongoing comprehensive pathological review and merging of epidemiological and pathological data will occur (Months 6-50).

Progress: This has been done for the master database and pilot.

Task 12. Complete merging and data management of Master data set (Months 48–50).

Progress: Completed and data is in review and being analyzed.

Task 13. Analysis of prospective data with respect to NSAIDs and OCP use will be completed: a) Associations between OCP/NSAID/Vitamin E use and prevalence of lesions will be evaluated, overall and stratified by BRCA mutation status as in Aim 1, and b) associations between use of these substances and molecular markers identified in Aim 2. Manuscripts for Aim 3 will be prepared and submitted. This aim will be informed from data generated in Aims 1 and 2 (Months 48-60).

Progress: We have completed our initial data review and analysis from both prospective and retrospective data regarding these medications. Given the low prevalence of STICs and low prevalence of this exposure in this population we will not undertake a prospective study.

Section III. Problem Areas of Project 5

There were no new challenges since last project report.

Section IV. Future Work in Project 5

The goal is to submit manuscripts for the epi study and the pilot study in Aim 1and work towards a manuscript on the molecular characterization of STICs.aim 2. We will also submit abstracts to AACR annual and ovarian meetings in 2017. We will also use this additional year to format the data from project 5 and the repository so it can be easily leveraged for future projects by our investigators or outside investigators upon request. We will publicize its existence at meetings and working groups.

Administration (Admin) Core, Biostatistics/Bioinformatics/Epidemiology (BBE) core, and Pathology (Path) Core

As in the past 4.5 years, the three cores have been integrated to each other; therefore, we combine their progress in this section to avoid reiteration. Because the tasks related to the three cores are relatively generic and are applicable for the entire research period, so we will rather report the specific progress related to the cores.

Section II. Progress to Date:

The Administration Core led by Drs. Kurman and Shih continues providing all the administrative support to all 5 research projects. Communications among project leaders have become a routine through several venues as described in previous reports. Specifically, we held regular meetings scheduled on the 1st and 3rd Tuesdays of each month to discuss issues, progress, questions etc. The Administration Core has help organized the face-to-face meeting of key personnel within this consortium through the venue of the annual meeting of the United States and Canadian Academy of Pathologists (USCAP) at Seattle Convention Center in March 2016. In addition to bi-weekly PI conferences at Hopkins, we also have had periodic teleconference with other site PIs. Special meetings were also held for Project 5 as it has generated substantial amount of new and very exciting data. The Project 5 investigators are working on manuscripts for 2 papers; a 400 case epidemiological study and 105 cases pilot study.

The BBE core provides excellent support to the current study design and assists statistical analysis for all the data generated in the past 6 months. The BBE core is currently working with investigators to prepare for manuscripts to be submitted to journal publications in the future.

The Path Core led by Drs. Visvanathan, Soslow, Kurman and Shih continues to serve as our central collection resource for the various projects. Highlights of Path Core progress this past period include:

<u>Path Core Activities since March 2016</u>

1- Supported ongoing efforts for sites with slide shipping, labeling, and data entry.
2- Continued to enter slides into the database upon receipt from sites. To date, > 46,000 slides have been entered and cataloged between Toronto, Yale, NYU and Hopkins (see table below). There are remaining slides to enter for Hopkins and we anticipate this will be competed in the next few weeks.
3- Coordinated a systematic slide cabinet storage check to ensure all slides properly integrated and organized.
4- Continued to trouble shoot site issues regarding use of the repository database.
5- Provided continued oversight of uploading of path-reports by all study sites. 6- Completed all slide staining for Project 5 pilot for each study site.

The past 6 months we have updated the database. All slides were entered to the database with 11 digit labels. Additional necessary immunohistochemistry was performed. The details of the case number are summarized below.

Breakdown of the slides at the biorepository is as follows,

Total Blocks and Slides: Breakdown by Institution

	JHM		MSKCC		Yale		Toronto		Total	
	Block	**Slide**	**Block**	**Slide**	**Block**	**Slide**	**Block**	**Slide**	**Block**	**Slide**
Ovarian Cancer Cases	42	2109	17	828	10	515	40	2346	109	5798
Prophylactic Cases	0	0	0	0	0	0	0	0	0	0

Prophylactic Project 5										
Cases	163	5313	141	5089	230	5956	355	24105	889	40463
Total	205	7422	158	5917	240	6471	395	26451	998	46261

Total Pathology Reports: Breakdown by Institution

	JHM	MSKCC	Yale	Toronto	Total
Ovarian Cancer Cases	29	15	0	25	69
Prophylactic Cases	0	0	0	0	0
Prophylactic Project 5					
Cases	96	152	44	198	490
Total	125	167	44	223	559

Total Cases: Breakdown by Institution

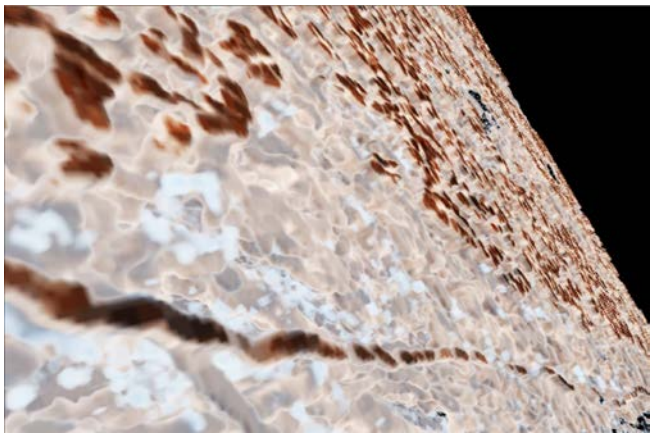
	JHM	MSKCC	Yale	Toronto	Total
Ovarian Cancer Cases	41	15	10	25	91
Prophylactic Cases	0	0	0	0	0
Prophylactic Project 5					
Cases	108	300	47	199	654
Total	149	315	57	224	745

Section III. Problem Areas for the Cores

So far, we do not expect major problems for the three cores.

Section IV. Future Works in Cores

Project 1 is collaborating with the Pathology Core to establish the 3-D reconstruction of ovarian cancer precursor and ovarian cancer using a new computer-based technology called Voloom (https://micro-dimensions.com/voloom/). This continuing task will allow investigators as well as physicians and patients to unprecedentedly view the relationship between ovarian cancer precursors in the fallopian tube and invasive carcinoma in a 3-D. This knowledge will become the road map to study STIC and ovarian cancer and we will present a video of this 3-D images to the public in the DoD Consortium website. Moreover, this 3-D images will help Project 1 to come up with the molecular landscape of STIC and p53 signatures by mapping the 3-D images with the molecular genetic findings. This will not only meet the objectives of the proposed task but will also create a new direction of research in years to come. For this, we have purchased the



software and will lease a high-performance slide scanner (Mikroscan, ~$4,000/month from 9/2016 to 9/2017 lease ends) to finish this task. One of the 3-D reconstruction of STIC and ovarian cancer is shown in the figure on the right. We will also use this scanner to archive important images from the tissues we collected in the Pathology Core. These high-quality of images will be stored in our secured server (provided by our institution without extra cost to this grant) and will be remotely accessible to all investigators for educational and research purposes through internet. Therefore, the no cost extension will allow the Pathology Core in collaboration with Admin Core to create not only a highly valuable tissue bank but also the image bank including the 3-D images (as one of the benchmarks of the DoD Consortium).

Resource Sharing Plan. Per the DoD OCRP guideline, the Administrative Core and Pathology Core will work together for resource sharing from those generated by the five projects through this consortium grant in the past 5 years. The resource to be shared with science community includes data, reagents, animal models and cell lines/primary cultures. The administrative core and the Biostatistics Core will be responsible to deposit the RNAseq and other related raw data, and the Pathology Core will gather and centralize a copy of aliquots of reagents and cell lines/primary cultures in our bank located at the main consortium site, the Johns Hopkins Medical Institutions. These resources will be then posted in our DoD website for public access. Upon request, the Consortium PIs and Project leaders will grant the approval within 4 weeks. The Administrative Core will then ship the granted materials after fulfilling the institutional regulatory guidelines (material transfer and IRB approvals).

Section V- Administrative Comments (Optional)

The no cost extension has been granted by DoD OCRP in October, 2016. This allows us to continue this consortium research and complete the tasks as proposed. Of note, Dr. Douglas Levine, the Project Leader in Project 2 has been recently relocated from MSKCCC to NYU Medical Center. As a result, the budget for Project 2 no cost extension will be transferred to NYU through the Administration Core.